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# 40th EASD Annual Meeting of the European Association for the Study of Diabetes

## Munich, Germany, 5–9 September 2004

# Abstracts

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# OP 1

# Diabetic foot

## 1

**Special pre-manufactured footwear with insoles can prevent ulceration in diabetic patients with diabetic foot syndrome by pressure reduction. A prospective randomised study**

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**Background and aims:** To prevent ulceration in diabetic foot syndrome (DFS) specially designed and manufactured shoes are offered to patients, but only very little is known about those shoes for preventing or treating DFS.
**Materials and methods:** 81 diabetic patients with significant sensory-motor and autonomic peripheral neuropathy without relevant peripheral artery occlusion disease were randomised and two kinds of diabetes-adapted shoes were selected: "comfortable shoes" ("CS") n=39 or "semi-orthopaedic shoes" ("SOS") n=42. For each pair of shoes a special kind of similar individually manufactured diabetes-adapted insole was made. The study was carried out over a period of 24.3 ± 1.8 months and every 3 months structured examination and documentation of shoes and feet were performed in the foot clinic (including pedography barefoot with emed®-platform) and also at the orthopaedic shoe manufacturer (within the shoes with emed-pedar®).
**Results:** 33% (13 out of 39) patients who were randomised to the "CS" group had to be switched to the "SOS" because of anatomical reasons. The dropout rate was 38% after 24 months and therefore 50 patients (16 in the "CS" group and 34 patients in the "SOS" group) remained under observation. 19 of the remaining 50 patients (38.0%) had a positive ulcer history but only 7 of them (14.0%) developed an ulcer during the trial, 4 of them (=21.1%, 4 out of 19) as an ulcer relapse. No ulcer relapse was seen in the group that was equipped with "CS" and one ulcer was observed in the patients who had to be switched to the "SOS". In the group using primarily "semiorthopaedic" shoes ("SOS") there was a re-ulceration rate of 15.0% after 1 year and of 28.6% after 2 years. This is much lower than in previous published trials concerning footwear supply for DFS. In all three groups there was a rate of new ulcers: 5.6%, 7.7% and 8.0% after 1 year and 6.3%, 9.1% and resp. 14.7% after 2 years ("CS","switching" and "SOS"). The plantar pressures were as follows:

| **Maximum pressure [N/cm2]** | barefoot 0year | barefoot 1year | barefoot 2years | in shoe 0 year | in shoe 1 year | in shoe 2 years |
|---|---|---|---|---|---|---|
| "comforable" | 39,00 ± 7,65 | 39,45 ± 7,09 | 41,75 ± 8,35 | 18,89 ± 4,19 | 21,52 ± 6,03 | 15,75 ± 1,75 |
| "semi-orthop" | 39,08 ± 8,39 | 41,48 ± 8,58 | 44,00 ± 8,21 | 19,31 ± 5,67 | 17,43 ± 3,80 | 16,83 ± 4,07 |
| **Pressure-time-integral [kPa * sec]** | | | | | | |
| "comfortable" | 27,35 ± 4,85 | 26,13 ± 6,79 | 30,31 ± 8,28 | 19,16 ± 4,18 | 17,47 ± 3,08 | 18,90 ± 0,90 |
| "semi-orthop" | 26,40 ± 7,53 | 26,97 ± 6,21 | 25,43 ± 5,30 | 18,56 ± 3,88 | 15,93 ± 3,57 | 15,73 ± 2,50 |

With both shoes and insoles there was a highly significant reduction ($p<0,02$) of the maximal pressure and the pressure-time-integral in comparison to barefoot. But there was no significant difference between the two shoe models and only minimal changes during the observational period.
**Conclusion:** With these premanufactured shoes the maximum pressure and the pressure-time-integral is significantly reduced with no significant difference between the two shoe models. It is sufficient for feet at "low risk" to supply patients with "comfortable" shoes ("CS") and for feet at "moderate or higher risk" it is better to supply them with "semiorthopaedic" shoes. In all patients there was no necessity for individually manufactured orthopaedic shoes, which are very expensive.

## 2

**The accuracy of incidence and prevalence of foot ulcers in diabetes – self-report vs medical records**

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**Background and aims:** In most conditions, disease recognition relies on screening, examination and self-reported data. The aim of this study was to assess the accuracy of self-reported ulcer prevalence and incidence from a sample of high-risk patients with diabetes.
**Materials and methods:** Patients were asked to report whether they ever had a foot ulcer at a baseline foot examination or developed a new foot ulcer during a follow-up assessment (mean 10 ± (sd) 2.1 months). Responses were compared with clinical records held at their local diabetes clinic and/or specialist foot clinic.
**Results:** 325 subjects were enrolled into the study and 230 (71%) attended the follow-up assessment and examination. 180/320 (78%) sets of medical records were compared. Baseline prevalence of an ulcer history recorded in the medical records was 65/320 (20.3%) with 75/320 (23.4%) subjects reporting a previous history of foot ulceration. [Sensitivity 0.87; 95% CI (0.78,0.93); Specificity 0.96; 95% CI (0.91,0.98)]; Positive Predictive Values (PPV) 0.92; 95%CI (0.83,0.96) and Negative Predictive Value (NPV) 0.94; 95% CI (0.88,0.97). At follow up, 33.5% said they had developed a new ulcer but only 22% had new lesions recorded in their notes [Sensitivity 0.85; 95% CI (0.72,0.92); Specificity 0.83; 95% CI (0.77,0.88)]. PPV at follow-up was 0.61; 95% CI (0.49,0.72) and NPV 0.94; 95% CI (0.89,0.97).
**Conclusion:** In spite of good sensitivity and specificity values, the proportion of patients who correctly reported the occurrence of a new ulcer was 61%. Although self-reported ulcers may be a reasonably accurate indicator of prevalence, the results suggest that additional methods of classifying and recording the occurrence of diabetic foot ulcers are essential in future studies if biased estimates of ulcer incidence are to be avoided.

## 3

**"Samadhan" – a new economical and effective technique for offloading body-weight in diabetic patients with neuropathic forefoot ulcers**

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**Background and aims:** Samadhan System (SS; Samadhan, Hindi word, meaning solution), a newly discovered method of offloading, is a workable solution to the problem of offloading. The present study aims to compare SS with Total Contact Cast (TCC). The SS needs merely a piece of foam (density 40), rolled into a cylinder, shape maintained with an adhesive lotion (Fevicol SR 998) applied over the inner side of the foam and placed over a place, which effectively renders offloading over the vulnerable part, retained in that position with the help of an elastocreppe band to which velcro and felt has been affixed.
**Materials and methods:** In a prospective, randomized, open label clinical study of 3 months duration, 30 middle aged (50 ± 4.24 years) type 2 Diabetes patients with almost similar duration of disease SSG=12.78 ± 3.16 and TCCG=15 ± 1.93 years) and glycemic control, judged on the basis of GHb A1 c (mean GHb A1 c=10.2%) with neuropathic fore foot ulcers (diagnosed with 10 point Semmes-Weinstein Monofilament)-grade I & II (Wagner's Classification) without Peripheral Ischaemia (Ankle Brachial Index >0.9; calculated with Kody's [Chennai, India based company] Hand Held Audio Doppler) and infection (ruled out with Swab Culture) were randomized, by draw of lot, to SS or TCC group.
**Results:** By the end of the study 86.6% ulcers healed in TCC group compared to 71% in SS group. Time period required for healing of ulcer in SS group was a little longer being 43.33 ± 17.50 days in comparison to TCC group 31.07 ± 10.95 days with p value of 0.050 and 95% CI -1.43 E-02 to 24.53. The average direct cost incurred on each patient in SS group was only rupees 50 (1 USD) compared to Rupees 3,900 (85 USD) in TCC group.
**Conclusion:** The SS of offloading, with its efficacy and economy, appears most affordable method of offloading.
**Abbreviations**: TCC; Total Contact Cast, SS; Samadhan System

## 4

**Differences in the predisposition to Charcot osteoarthropathy in Type 1 and Type 2 diabetes**

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**Background and aims:** Controversy exists as to the importance of diminished bone density and the type of neuropathy in the predisposition to Charcot osteoarthropathy (COA). However, such predisposition may be different in type 1 and type 2 diabetic patients. The aim of this study was to compare calcaneal bone mineral density and the type of neuropathy between type 1 and type 2 patients with COA and to compare these parameters with type 1 and type 2 diabetic control patients of similar age and duration of diabetes. We specifically studied the non-Charcot foot to assess bone density and nerve function that existed at the onset of COA.
**Materials and methods:** We studied 17 type 1 and 18 type 2 patients presenting with recent onset of COA. Controls were 47 type 1 and 48 type 2 diabetic patients. Bone mineral density (BMD) was measured by quantitative ultrasound and expressed as Z-score in standard deviations (SD). Both small fibre (temperature perception threshold: TPT-hot and TPT-cold) and large fibre neuropathy (vibration perception threshold: VPT) were assessed. Parameters of the non-Charcot foot in type 1 COA patients were compared with type 2 COA patients. Type 1 and type 2 COA patients were also compared with type 1 and type 2 controls respectively.
**Results:** In type 1 COA patients, BMD in the non-Charcot foot was significantly reduced compared with type 2 COA patients (Z-score:-1.6 ± 0.9 SD vs -0.6 ± 0.8 SD, $p<0.001$). In type 1 COA patients BMD of the non-Charcot foot was also reduced compared with type 1 controls (Z-score:-1.6 ± 0.8 SD vs -0.6 ± 0.8 SD, $p<0.001$). In contrast, in type 2 diabetes, there was no difference between BMD of the non-Charcot foot compared with controls (Z-score: 0.02 ± 0.9 SD vs 0.2 ± 0.9 SD, p=NS; Non-significant).
As to small fibre neuropathy, both type 1 and type 2 COA patients had considerable but similar impairment to hot (12 ± 4.7°C vs 12 ± 4.3°C, p=NS) and cold (12 ± 4.2°C vs 9.5 ± 4.2°C, p=NS in the non-Charcot foot. Furthermore, this impairment to hot and cold was significantly greater in type 1 COA patients versus type 1 controls (TPT-hot: 12 ± 4.7°C vs 8.5 ± 4.6°C, $p<0.01$; TPT-cold: 12 ± 4.2°C vs 7 ± 4.7°C, $p<0.01$) and it was also significantly greater in type 2 COA patients versus type 2 controls (TPT-hot: 12 ± 4.3°C vs 7.5 ± 4.3°C, $p<0.01$; TPT-cold: 9.5 ± 4.2°C vs 5.5 ± 1.4°C, $p<0.01$).
In contrast, large fibre neuropathy was more common in the type 2 COA patients compared with type 1 COA patients: 94% of them had VPT greater than 25 volts compared to 62.5% of the type 1 COA patients ($p<0.05$). Indeed, there was no significant difference between the VPT in the type 1 COA patients and controls (29 ± 12.3 volts vs 22 ± 15.2 volts, p=NS), in contrast to the type 2 COA patients in whom VPT was significantly higher compared with controls (40 ± 10.2 volts vs 21 ± 4.3 volts, $p<0.001$).
**Conclusion:** This study has shown that there was a difference in the clinical predisposition to COA in type 1 and type 2 diabetes. There was an underlying osteopenia as indicated by reduced BMD in the non-Charcot foot in type 1 but not in type 2 diabetes. Small fibre neuropathy was detected in both type 1 and type 2 COA patients in contrast to large fibre neuropathy which was mainly found in type 2 COA patients. These observations may explain the controversy regarding bone density and type of neuropathy in previous studies of COA, which have not differentiated between type 1 and type 2 diabetes. In future studies of COA, these patients should be described separately.
*This study was funded by Diabetes UK.*

## 5

**Importance of distal PTA in the treatment of neuroischaemic diabetic foot**

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**Background and aims:** Neuroischeamic diabetic foot represents a serious clinical condition due to very frequent involvement of distal arteries in the obstructive vascular disease. This complication is strictly correlated with major amputation in diabetic population. The aim of our study is to value the role of PTA, as first revascular procedure, in the treatment of neuroischeamic diabetic foot with the goal of limb savage.
**Materials and methods:** in a period January 2001 to November 2003 we have consecutively enrolled and submitted to angiographic evaluation 243 legs and contemporary PTA in 222 patients (137 M 106 F mean age 70 years) affected by foot ischeamia with skin lesions (gangrene or ulcer involving the foot) with pedal pulses absent and with TcP02≥ 40 mm Hg. PTA was considered effective in case of complete vessel recanalization with direct flow obtained from the iliac-femoro-popliteal segment in at least one vessel of the foot. The primary end point of the study was: 1) limb savage with reduction of major amputation 2) increase in TcP02 level.
**Results:** out of 243 legs submitted to angiographic evaluation in 220 cases (90%) was possible to perform a PTA with 449 obstructive lesions treated (mean lesions treated per procedure = 2 with range from 1 to 4). The characteristics of the treated district were: 1) iliac-femoro-popliteal 197 patients (44%) 2) infrapopliteal segments 252 patients (56%). The mean length of a single lesion treated was 9 cm while mean length of vessel treated per procedure was 18 cm. Pta was considered effective in 206 patients out of 243 treated (93%). We observed an increase in TcP02 level from 23 ± 14 before PTA to 46 ± 14 after PTA. We obtained the limb savage in 209 patients out of 243 patients submitted to PTA . We observed only local complication due to femoral arterial puncture in 4% of the patients corrected with conservative treatment.
**Conclusion:** data coming from our study indicate that PTA should be considered a safe and efficacy procedure in the treatment of critical ischemia in diabetic foot. The association between endoluminal vascular procedure and precocious and correct surgical approach is able to obtain the reduction of major amputation in diabetic population.

## 6

**A randomized trial of two irremovable offloading devices in the management of plantar neuropathic diabetic foot ulcers**

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**Background and aims:** Offloading of plantar neuropathic foot ulcers is fundamental to successful management. While the total contact cast (TCC) is the gold standard, it is infrequently used due to lack of expertise, cost and fear of complications. Removable cast walkers (RCW) offload as effectively as TCCs, but fail to produce equivalent healing rates either in practice or randomized trials.
**Materials and methods:** We undertook a randomized controlled study to compare treatment with the standard TCC with the DH RCW (Royce Medical, CA), rendered irremovable by wrapping it with a single strip of fiberglass cast material, thus converting it into an irremovable instant total contact cast (iTCC). Forty one consecutive patients, 21 in the TCC and 20 in the iTCC, with non-infected, non-ischemic, plantar neuropathic foot ulcers, were studied. Patients meeting inclusion criteria and felt by the treating physician to be candidates for casting were approached about study participation. Informed consent was obtained, the patients were screened and then randomly assigned, using a random number table, to either group. They were then followed until the ulcer healed or 12 weeks.
**Results:** The mean patient age was 50.9 years. The average ulcer duration was 216 days (median 68 days). There were no demographic differences between the two groups. No differences in times to healing by Kaplan-Meier analysis ($P>0.9$) were observed (TCC median 5 weeks vs. iTCC median 4 weeks). Nor were there differences in all complications (65% in TCC group vs. 38% in iTCC group, $P=0.09$) with 54% of the complications in the TCC group and 68% in the iTCC group being excessive skin moisture. Other complications were rare. One patient in each group required amputation of a single toe. The amputation in the patient assigned to the iTCC was directly related to patient non-compliance and in the patient wearing the TCC it was a result of a cast induced ulcer. The most striking finding was the time of cast application and removal. Application times were: TCC 12 m25s vs. iTCC 7 m38s ($P<0.0001$). Removal times were: TCC 3 m36s vs. iTCC 2 m17s ($P<0.0001$).
The resulting direct costs of cast therapy in the TCC group were $38.36 weekly for materials and in the iTCC group a one time cost of $89.95 for a RCW and $14.70/week in materials. The total cost of treating a single patient in the TCC group was $210.67 vs. $158.47 in iTCC group.
**Conclusion:** We conclude that the iTCC is equally efficacious in healing plantar neuropathic ulcers, when compared to the TCC. However, it is quicker, easier, and more cost effective than the TCC. If larger studies confirm our findings, it will make effective offloading universally available without the need for highly skilled technicians, increase patient access to care, reduce health care costs, possibly decrease complications and potentially revolutionize the treatment of diabetic plantar neuropathic ulcers.

# OP 2

# Insulin: alternative approaches

## 7

**Pharmacodynamics and pharmacokinetics of dose ranging effects of Oralin versus s.c. regular insulin in healthy volunteers**

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**Background and aims:** The purpose of the study was to evaluate the pharmacodynamic and pharmacokinetic properties and the dose ranging effects of a buccal spray insulin formulation (Oralin) in comparison to s.c. regular insulin as well as to placebo spray in healthy subjects.
**Materials and methods:** In this randomized, 5-way, crossover study, 7 healthy volunteers were assessed over 5 test periods, 7 days apart and received 4 different doses of buccal spray: spray insulin: 5, 10 and 20 puffs, placebo spray – 10 puffs and a dose of 0.1 U/kg s.c. regular insulin, under euglycemic clamp for 6 hours after administration. Somatostatin was used in order to inhibit endogenous insulin and glucagon secretion.
**Results:** Oralin had an earlier onset of action (Time to early half-maximal effect: 31.7 ± 12 vs 77.8 ± 28 min, $p < 0.05$), earlier peak (Time to maximal effect: 44.2 ± 10 vs 159.2 ± 68 min, $p < 0.05$) and a shorter duration of action (Time to late half-maximal effect: 85.1 ± 25 vs 319.2 ± 45 min, $p < 0.05$) compared with s.c. insulin. The maximal metabolic effect (GIRmax: 1.72 ± 1.03, 3.09 ± 1.68, 4.61 ± 1.53 mg/kg/min, $p < 0.05$) and the amount of glucose infused (GIR-$AUC_{0-120}$: 106.69 ± 74.3, 162.87 ± 116.1, 253.99 ± 122.92 mg/kg/120 min and GIR- $AUC_{0-360}$ :399.6 ± 205, 456.74 ± 194, 569.46 ± 239 mg/kg/360 min) increased in a dose-dependent relationship for the three doses of oral insulin. The amount of glucose infused after administration of subcutaneous insulin was: GIR- $AUC_{0-120}$:305.56 ± 150 mg/kg/120 min and GIR- $AUC_{0-360}$ :1340.16 ± 321 mg/kg/360 min.The time to maximum insulin concentration was shorter for the Oralin compared to s.c insulin (25.9 ± 9 vs 145.7 ± 49 min, $p < 0.05$). The maximum insulin levels were comparable in the s.c. vs 20 puffs of Oralin (39.1 ± 19.63 vs 34.03 ± 7.49 µU/ml/120, p-NS). The AUC for serum insulin (Ins-$AUC_{0-120}$ :339.82 ± 218, 681.38 ± 407 and 1586.78 ± 824 µU/ml/120 min, respectively, $p < 0.05$ and Ins- $AUC_{0-360}$ :831.07 ± 384, 1464.7 ± 775 and 2423.57 ± 1409 µU/ml/360 min, $p < 0.05$) and maximum insulin levels (Ins max: 7.57 ± 2.8, 16.43 ± 9.3 and 39.1 ± 7.49 µU/ml, respectively, $p < 0.005$) proved a dose response relationship for the 3 doses of spray insulin. After administration of subcutaneous insulin, the AUC for serum insulin were: Ins- $AUC_{0-120}$:2529.73 ± 725 µU/ml/120 min and Ins- $AUC_{0-360}$ : 7571.87 ± 815 µU/ml/360 min.
**Conclusion:** Oralin was absorbed in a direct relation with the amount given, had a faster onset and a shorter duration of action compared to s.c. regular insulin. A dose-response relationship in the absorption and metabolic effect of the Oralin was noticed.

## 8

**Oral insulin as first-line therapy in Type 2 diabetes: a randomized-controlled pilot study**

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**Background and aims:** Preprandial s.c. insulin has been shown to be efficacious, albeit difficult to titrate in subjects with early type 2 diabetes but many patients are reluctant to use s.c. injections as first-line therapy. Therefore, we investigated the safety and efficacy of treatment with an oral insulin (OI) formulation over two weeks in subjects with type 2 diabetes well-controlled under dietary conditions in a randomised, double-blind, placebo-controlled, parallel-group pilot study.
**Materials and methods:** Thirteen subjects with diet-controlled type 2 diabetes (11 males, 3 females, age 59 ± 9 years (mean±SD), HbA1 c 6.6 ± 0.7%, BMI 29 ± 3 kg/m$^2$) received either 2 tablets of with altogether 300 IU insulin + 160 mg carrier or 200 mg carrier alone four times a day (10 min before meals and at bedtime) over 14 days. Efficacy of OI was assessed under in-house conditions at baseline and at the end of treatment by blood glucose (BG) and insulin (INS) profiles under standardized meal conditions, and during oral glucose tolerance tests (OGTT). In addition, insulin resistance measurements (HOMA-IR), pharmacokinetic (PK) and fructosamine determinations were performed. Safety was assessed by collection of adverse events, safety laboratory, and clinical examinations.
**Results:** Compared with baseline values, treatment with OI improved glycaemic control, indicated by lower end of treatment values for BG-AUCs under both standardized and OGTT conditions, and by lower fructosamine concentrations (table). These changes were associated with improvements in insulin sensitivity and, consecutively, with reductions in postprandial insulin concentrations. All these improvements were greater than those observed in the control group, although no statistical comparisons are provided due to the exploratory nature of this study. Treatment with OI was well tolerated, only 2 mild to moderate adverse events (arthralgia, headache) occurred compared with 3 events in the control group. No hypoglycaemic events were observed despite the tight glycaemic control of the patients.
**Conclusion:** The results of this pilot study in well-controlled subjects with type 2 diabetes show that treatment with oral insulin is well-tolerated and improves both glycaemic control and insulin sensitivity. Insulin delivered portally may have distinct advantages as compared to s.c. insulin. Among the attributes are reduced postprandial insulin concentrations and thus less risk of hypoglycaemia, and it might help to preserve β-cell secretion capacity. In view of these promising results, larger and longer trials are warranted to investigate the safety and efficacy of oral insulin as first-line therapy in early type 2 diabetes.

| | Mean changes (±SD) | | % change | |
|---|---|---|---|---|
| | Oral Insulin | Control | Oral Insulin | Control |
| BG-$AUC_{0-1\,h}$ (OGTT) [mg*h/dL] | −57 ± 44 | −16 ± 33 | −22 | −8 |
| BG-$AUC_{total}$ (standardised diet) [mg*h/dL] | −335 ± 250 | −184 ± 206 | −19 | −11 |
| INS-$AUC_{0-1\,h}$ (OGTT) [µU*h/mL] | −9.9 ± 21.0 | 0.8 ± 10.2 | −17 | 2 |
| INS-$AUC_{total}$ (standardised diet) [µU*h/mL] | −39 ± 168 | −23 ± 119 | 1 | −7 |
| Insulin resistance (HOMA-IR) | −52 ± 57 | −14 ± 42 | −37 | −16 |
| Fructosamine [µmol/L] | −21.7 ± 7.2 | −17.5 ± 30.4 | −9 | −6 |

Table gives mean changes (±SD) and percent changes of end of treatment vs. baseline. Total AUCs are the sum of the 3-hour postprandial AUCs after each meal during the in-house days.

## 9

**An international trial of sulfonylurea plus either metformin or Exubera®: impact on quality of life and treatment satisfaction**

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**Background and aims:** Because of the burden of daily injections, insulin therapy in type 2 diabetes is often initiated only after failure on multiple oral agents. Prior to switching to insulin, many individuals are in suboptimal glycemic control. To assess quality of life (QOL) and patient satisfaction with diabetes treatment when initiating insulin earlier using pulmonary delivery with Exubera®, we studied 423 type 2 diabetes subjects from Europe, Africa, Asia and South America, poorly controlled on sulfonylurea monotherapy, and randomized to adding either premeal Exubera®, n = 222 or metformin, n = 201 for 24 weeks.
**Materials and methods:** Randomization was stratified (HbA1 c = 8.0–9.5% and > 9.5–12.0%) and medications titrated to goal fasting plasma glucose of 4.4–7.8 mmol/l. Self-administered questionnaires were completed at Weeks 0 (Baseline), 10, 18, 24 and Exit, and included measures of QOL (subscales of physical, emotional and social functioning) and satisfaction with diabetes medication (subscales of advocacy, burden, convenience, efficacy, flexibility, general satisfaction, preference and side effects). Eighty-eight percent of the eligible subjects (373/423) met the intent to treat analysis requirements for completing both a valid baseline and at least one follow-up questionnaire. In addition, 357 of those 373 subjects (95.7%) completed the Week 24 questionnaire.
**Results:** Patients were 53% male and had mean±SD age = 60 ± 9 yrs, BMI = 28.8 ± 4.4 kg/m2, and HbA1 c = 9.7 ± 1.0%. Endpoint HbA1 c±S.E. was lower for Exubera® (7.6 ± 0.06%) vs metformin (7.8 ± 0.07%), P=0.025. Study dis-

continuation was greater for metformin (11%) than Exubera® (6%), P=0.04. Pooling strata, Overall QOL (in SD units) improved similarly for Exubera® (by 0.24± 0.09, P=0.01) and metformin (0.21± 0.11, P=0.06). Overall Satisfaction (scaled 0–100) improved substantially and similarly for Exubera® (62.1 to 76.1, P = 0.0001) and metformin (63.1 to 74.1, P=0.0001). However, endorsement (eg., recommendations to family and friends) for treatment increased more for Exubera® (61.3 to 90.2) than metformin (64.3 to 81.2), P=0.005. Somewhat more dissatisfaction with side effects (weight gain and hypoglycemia) was found for Exubera® (83.1 to 77.2) vs metformin (82.1 to 81.2), P=0.047. There was no change in burden or convenience. For stratum HbA1c > 9.5%, endpoint HbA1c was lower for Exubera® (7.8±0.09%) than metformin (8.3±0.12%), P=0.01, and improvement in Overall Satisfaction was 42% greater for Exubera® (602 to 771) than metformin (612 to 732), P=0.02. Improvements in HbA1c were positively correlated with improvements in Overall Satisfaction (r = 0.254, p < 0.0001).
**Conclusions:** In this international trial, subjects with HbA1c between 8 and 12% showed superior glycemic control and lower discontinuation with Exubera® compared to metformin, while changes in quality of life and treatment satisfaction were similar. However, for those with HbA1c > 9.5,%, superior efficacy with Exubera® and neutral quality-of-life burden translated into higher overall satisfaction with diabetes treatment as compared to metformin. Patient acceptance and satisfaction with Exubera®'s pulmonary delivery of insulin may allow many persons failing on combination oral agents to add or switch to insulin earlier.

## 10

**Glucose-modulated insulin gene therapy for diabetes**
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**Background and aims:** Insulin-dependent diabetes mellitus (IDDM) results from impairment of the insulin-producing pancreatic β-cells. The insufficiency of insulin secretion often results in several secondary complications. Here in this study, recombinant adeno-associated virus (rAAV) was employed as a gene delivery vector for the glucose-modulated gene therapy in streptozotocin (STZ)-induced diabetic animals.
**Materials and methods:** Recombinant AAV, containing a furin mutated human insulin gene, driven by the rat insulin I promoter, was constructed. Huh7 human hepatoma cell was employed to investigate the glucose responsiveness *in vitro*. Effect of dbcAMP, theophylline, and foskolin on human insulin production in the rAAV-transduced Huh7 cells was determined by radioimmunoassay (RIA). The STZ-treated diabetic C57BL/6J mice were used for *in vivo* studies. Recombinant AAV was injected into the liver parenchyma of the animals, and the blood glucose levels were monitored and human insulin secretion in the animals was determined by RIA.
**Results:** Increasing glucose concentrations elevated insulin secretion in the Huh7 cells, with a maximal induction at 20 mM glucose. Addition of dbcAMP, forskolin, and theophylline modulated insulin secretion *in vitro*. The *in vivo* experiments also demonstrated good correlation between insulin secretion and blood glucose levels in the STZ-induced diabetic animals, and hyperglycemia was corrected in those animals treated with rAAV. The glucose responsiveness was confirmed by glucose tolerance test.
**Conclusion:** Recombinant AAV was employed for hepatic gene therapy in STZ-induced diabetic animals in the present study. Glucose and insulin secretagogues were shown to modulate the transgene expression in rAAV-transduced hepatoma cells, suggesting that conditions affecting insulin expression in pancreatic islet beta cells also affect transgene expression in human hepatoma cells conferred with insulin gene promoter. Results obtained from the *in vivo* experiments demonstrated that glucose-modulated transgene expression in STZ-treated diabetic animals was obtained after treatment with rAAV.
*Supported by: National Science Council, Taiwan*

## 11

**The gene expression pattern and morphology of mouse embryonic stem cells during differentiation into insulin-producing cells**
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**Background and aims:** Embryonic stem cells present an unlimited source of cells, which have the potential to differentiate into tissues from all three germ layers. The shortage of transplantable donor islets of Langerhans for therapy of type 1 diabetes mellitus has focused research on the development of surrogate cells with characteristics similar to those of pancreatic beta cells. The aim of this study was to examine the gene expression and the morphology of mouse embryonic stem cells using an improved differentiation protocol, which enriches nestin positive cells as putative pancreatic progenitor cells.
**Materials and methods:** Mouse embryonic stem cells (mESC) from the D3 cell line were differentiated with a modified so-called Lumelsky protocol. In different stages of this differentiation protocol the gene expression of a wide range of genes was examined by quantitative real-time PCR analyses. The differentiation stages were in addition also studied by electron microscopy (EM). Apoptosis was assessed during the differentiation process through quantification of caspase-3 expression as well as caspase-3 enzyme activity.
**Results:** In the initial stages the cells were Oct4 and Pdx1 positive, but showed no significant expression of insulin and nestin. These cells exhibited typical signs of undifferentiated stem cells revealed by EM studies. In stages 2–3 many cells showed neuronal characteristics. In stage 5 the cells showed characteristics of insulin producing cells. The cells expressed nestin and insulin mRNA, but also mRNA for glucagon and somatostatin. In ultrastructural analyses stage 5 cells showed rough endoplasmic reticulum and Golgi apparatus structures as an indication of protein biosynthesis and to a reproducible degree signs of endocrine differentiation. In addition the stage 5 cells expressed Kir6.2 and Sur1. Glut2 and Pdx1 gradually decreased during the differentiation but recovered when the medium in stage 5 was supplemented with FCS, providing an explanation for cell death of pancreatic progenitor cells under serum free conditions. Around 10% of the cells were necrotic or apoptotic in the initial differentiation stages increasing to one third in the final stage. The majority of these cells were apoptotic. Caspase-3 activity increased two-fold from the first stage to the last stage, with a peak in stage 3.
**Conclusion:** It could be shown that this protocol is able to drive differentiation of mESC towards cells with characteristics of insulin-producing cells but that special attention is required with respect to the optimization of differentiation conditions. It seems that besides the genetic approach through overexpression or gene trapping to enhance the differentiation of mESC towards insulin producing cells an improved cell culture protocol might also solve the problems of inadequate differentiation and loss of viability.
*Supported by: the European Union in the 5th FP (project QLRT-2001-01777).*

## 12

**Isolation and structural characterisation of a novel 13 amino acid insulin-releasing peptide from the skin secretion of Agalychnis calcarifer**
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**Background and aims:** The discovery of bioactive peptides in the skin secretions of frogs of the Phyllomedusinae subfamily has increased the focus on other members of this family such as *Agalychnis* as a source of pharmacologically active peptides. The aims of the present work were to isolate and characterise novel insulinotropic peptides from the skin secretions of *Agalychnis calcarifer* frogs.
**Materials and methods:** Crude secretions (50 mg; 5–10 frogs) obtained by mild electrical stimulation from the dorsal skin surface were purified by reversed-phase HPLC on semipreparative Vydac C18 column yielding 80 fractions. These fractions were assayed for insulin-releasing activity using glucose responsive BRIN-BD11 cells. Acute 20 min incubations were performed in Krebs Ringer bicarbonate buffer supplemented with 5.6 mmol/l glucose in the absence (control) and presence of various fractions (n=3).
**Results:** Fractions 20–21 (band 1), fractions 35–45 (band 2) and fraction 53 (band 3) showed significant 1.6–6.6-fold increase in insulin releasing activity ($p<0.001$, n=3) compared with control. The peaks showing insulin-releasing activity were subsequently purified by HPLC to single homogenous peaks. The fractions in band 1 (20–21) were pooled rechromatographed and peaks 1.1-1.20 were hand collected. Band 2 (fractions 35-45) was also purified further yielding peaks 2.1-2.5. There was a 1.3-2.7-fold increase in insulin release from BRIN-BD11 cells with peptides corresponding to peaks 1.3, 1.10, 1.17, 1.18, 2.4 and 2.5 compared with 5.6 mmol/l glucose alone ($p<0.001$, n=3). The final purification revealed a most potent peptide peak (1.10) with insulin-releasing activity and non-toxic effects on BRIN-BD11 cell viability as assessed by neutral red after acute incubations (20 min). Structural analysis of the peptide peak 1.10 was performed by mass spectrometry and automated edman degradation. This revealed a novel insulinotropic 13 amino acid 1653.2 Da peptide with the primary structure of RRKPLFPLIPRPK (RK-13). The data base search for the novel insulin releasing peptide showed a 53.8% homology with the N-terminal of the proline-arginine rich antimicrobial peptide ((PR-39), originally isolated from pig intestine). Preliminary studies on the mechanism of action of the RK-13 revealed that the insulin releasing effect was not abolished by co-

incubation with 50 µmol/l verapamil (verapamil vs RK-13; 1.74 ± 0.16 vs 2.75 ± 0.12 ng/$10^6$/20 min, P<0.05, n=8) and was clearly evident in cells depolarized with 30 mmol/l KCl (KCl vs RK-13; 5.32 ± 0.04 vs 16.45 ± 0.10 ng/$10^6$/20 min, P<0.001, n=8). Overnight culture of BRIN BD11 cells with 10 nmol/l PMA or 0.1 µg/ml pertussis toxin did not abolish the insulin releasing ability of RK-13 (P<0.001, n=8).
**Conclusion:** The study has revealed that the skin secretions of *Agalychnis calcalifer* frogs contain novel insulin-releasing peptides, including RK-13, which will prove useful for further investigation as insulin secretagouges and potential antidiabetic drugs from natural sources.

# OP 3

# Pregnancy in Type 1 diabetes

## 13

**Pregnancy outcomes in Type 1 diabetes: Can we ever get pregnancy outcomes comparable to the non-diabetic population?**
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**Background and aims:** Women with type 1 diabetes can encounter severe adverse outcomes such as an increased risk of abortions, foetal death, neonatal death and congenitally malformed babies. The St Vincent Declaration of 1989 set us a five year target that " the outcome of diabetic pregnancy should approximate that of the non-diabetic pregnancy". Large studies from the United Kingdom in the early 1990's showed that we are far from achieving these targets in unselected populations of pregnant women with type 1 diabetes. However, the Diabetes control and complications trial has shown that timely institution of intensive insulin therapy for glycaemic control in pregnant women with type 1 diabetes, can be associated with pregnancy outcomes comparable to normal population rates. Most centres in the United Kingdom strive to achieve good pregnancy outcomes in joint antenatal clinics by implementing strategies to improve glycaemic control although the uptake of pre-pregnancy counselling is not universal. However, by adopting such a strategy, there may be an increased risk of maternal morbidity (Caesarean sections, hypoglycaemic events and retinopathy) and foetal morbidity (hypoglycaemia, prematurity and neonatal unit admissions). Hence, we planned this comprehensive audit to look into the morbidity experienced by pregnant women with type 1 diabetes and in the neonates of these women in addition to mortality and other outcomes.
**Materials and methods:** Prospective audit of 176 pregnancies (157 women with type 1 diabetes) between 2000–2002 from the Mid-Trent region, England. Outcome data (Foetal mortality, perinatal and maternal morbidity) were collected from 165 completed datasets.Background population data (England & Wales) was extracted from the Office for National Statistics (200%02) and data from the National Congenital Anomaly System (2000–01) to calculate the relative risks for perinatal mortality and congenital malformations respectively.
**Results:** There were 138 live births (83%) including a twin delivery, 16 spontaneous (10%) and 8 induced abortions (5%), 2 intrauterine (1%) and 2 neonatal deaths (1%). The Perinatal Mortality rate was 21 per 1000 total births [relative risk (CI) - 2.61 (0.85- 8.01), p > 0.05]. There were 11 major congenital anomalies [67 per 1000 pregnancies, relative risk (CI)-5.24 (2.96-9.27), p < 0.001]. 69 (50%) were macrosomic babies with birth weight above the 95$^{th}$ centile ( Mean ± SE birth weight was 3352.2 ± 123.5 grams). 47 neonates required admission to special care baby unit (34% of live births). There were 90 caesarean sections (55%). 36 mothers had severe hypoglycemic events (22%), 12 developed retinopathy (7%), 7 developed proteinuria (4%), 15 developed pregnancy induced hypertension ( 9%) and 3 developed diabetic ketoacidosis (2%). Pre-pregnancy counselling was taken up by 65 women (39%). Mean ± SE $HbA_1C$ at booking and term were 8.3% ± 0.11 and 6.99% ± 0.19 respectively. Most of the babies with malformations were born to mothers with poor glycaemia in the periconceptive period.
**Conclusion:** Adverse pregnancy outcomes in women with type 1 diabetes from unselected populations considerably exceed those in normal pregnancy despite improvements in antenatal care. Although advances in foetal monitoring and neonatal care have reduced perinatal mortality rates, congenital malformations remain an important cause of mortality and morbidity.The prevention of adverse outcomes will depend on novel systems of pre-pregnancy care in women with diabetes.
*Supported by: Mid-Trent Diabetes in Pregnancy Group*

## 14

**Plasma concentrations of vascular adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) are increased early in pregnancy in type 1 diabetic women who subsequently develop preeclampsia**
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**Background and aims:** The incidence of preeclampsia in pregnancy is 2–4 fold increased in women with type 1 diabetes as compared to non-diabetic

women. The pathophysiological mechanism is unknown but as in non-diabetic women, impaired placental perfusion resulting in release of substances toxic to the maternal vasculature is hypothesized. In diabetes, risk factors for preeclampsia include hypertension, microalbuminuria and elevated plasma homocysteine thereby resembling risk factors for atherosclerosis. Adhesion molecules; VCAM-1, ICAM-1 and E-selectin are expressed on endothelial cells and leucocytes in response to atherogenic stimuli mediating leucocyte adhesion to the endothelium followed by leucocyte transendothelial migration. The plasma concentrations of soluble forms of these molecules are previously reported elevated in established preeclampsia. The aim of the present study was to test the hypothesis that the plasma concentrations of soluble VCAM-1, ICAM-1 and E-selectin are elevated in pregnant women with type 1 diabetes who subsequently develop preeclampsia

**Materials and methods:** Eighty-five consecutive pregnant women with type 1 diabetes and a diabetes duration of more than 10 years were enrolled and plasma concentrations of adhesion molecules were measured in gestational weeks 11 and 28 by ELISA methods. Results on adhesion molecule concentration measurements of 82 (week 11) and 73 (week 28) women are reported.

**Results:** Fourteen women developed preeclampsia and were compared with the 71 women who did not. Plasma concentrations of VCAM-1 and ICAM-1 were significantly higher in week 11 in women who later developed preeclampsia than in women who did not (preeclampsia group vs. no preeclampsia group, 612 ± 82 vs. 507 ± 104 ng/mL, $p<0.001$ and 293 ± 67 vs. 256 ± 57 ng/mL, $p<0.05$, respectively). No significant differences were found in the concentrations of VCAM-1 and ICAM-1 in week 28 (581 ± 103 vs. 516 ± 117 ng/mL, $p=0.10$ and 273 ± 44 vs. 256 ± 41 ng/mL, $p=0.24$, respectively) and plasma concentrations of E-selectin were not different between the two groups either in week 11 or in week 28.

**Conclusion:** Plasma concentrations of VCAM-1 and ICAM-1 are higher in gestational week 11 in pregnant women with type 1 diabetes who later in their pregnancy develop preeclampsia than in women who do not. No significant differences are seen in gestational week 28. This may indicate that the higher concentrations reflect a vascular susceptability of the women prone to preeclampsia and that it is not a result of a subclinical part of the preeclamptic process.

## 15

### Circulating endothelial cell adhesion molecules in women with gestational diabetes and Type 1 diabetes

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**Background and aim:** Diabetic pregnancy is associated with vascular damage and endothelial dysfunction. Concentrations of circulating cell adhesion molecules are increased in diabetes and preeclampsia.
We measured levels of soluble vascular cell adhesion molecule sVCAM-1 and E-selectin in pregnant women with type 1 and gestational diabetes (GDM).

**Materials and methods:** We studied pregnant women with type 1 diabetes (n=18), and healthy pregnant women (n=20) during three trimesters [T1, T2, T3] and 6-10 weeks postpartum. Women with GDM (n=13) were studied during T3 and postpartum. ELISA method was used to quantitate concentrations of sVCAM-1 and E-selectin.

**Results:** Plasma levels of sVCAM-1(in ng/ml) and E-selectin(in ng/ml) were significantly higher in women with GDM during T3 compared with healthy pregnant controls [sVCAM-1: 878.44 ± 97.18 vs 582.72 ± 41.95 (mean ± SEM), p=0.009; E-selectin: 16.18 ± 2.75 vs 9.12 ± 0.77, p= .034]. E-selectin [26.73 ± 5.51vs 10.70 ± 0.99 , p=0.001] and sVCAM-1 levels [1108.25 ± 194.76 vs 633.87 ± 42.25, p=0.040] remained significantly increased postpartum in women with GDM. None of the women with GDM developed preeclampsia, although one of the healthy controls did and this subject was excluded from further analysis, as values of cell adhesion molecules were more than three standard deviations above control means. In contrast, concentrations of sVCAM-1 and E-selectin were lower in women with type 1 diabetes compared to controls, which was significant in T2 [sVCAM-1: 437.25 ± 27.59 vs 611.06 ± 48.6, p=0.01;E-selectin: 8.09 ± 0.73 vs 10.91 ± 0.7, p=. 012]. Five women with type 1 diabetes developed preeclampsia. sVCAM-1 concentrations were higher in the preeclamptic group during pregnancy and postpartum. E-selectin levels continued to remain at a similar level in the preeclamptic group during pregnancy but there was a decreasing trend in non-preeclamptic group.

| | | sVCAM-1 (ng/ml) | | E-selectin (ng/ml) | | |
|---|---|---|---|---|---|---|
| Trimester | Type1 diabetes-non preeclamptic (n=13) | Pre-eclamptic (n=5) | p | Type1 diabetes-non preeclamptic (n=13) | Pre-eclamptic (n=5) | p |
| T1 | 445.94 ± 68.29 | 669.08 ± 140.73 | 0.005 | 15.48 ± 2.19 | 10.53 ± 2.30 | 0.005 |
| T2 | 410.474 ± 35.57 | 496.90 ± 28.63 | 0.005 | 7.66 ± 0.88 | 9.18 ± 1.39 | 0.005 |
| T3 | 526.17 ± 24.27 | 610.15 ± 67.31 | 0.002 | 7.15 ± 0.89 | 9.92 ± 2.20 | 0.005 |
| Postpartum | 351.09 ± 89.7 | 628.22 ± 94.47 | 0.002 | 10.19 ± 0.95 | 8.79 ± 0.47 | 0.011 |

**Conclusion:** Circulating concentrations of sVCAM-1 and E-selectin are increased in women with GDM compared with healthy pregnancies. Elevated levels of these substances postpartum suggest that there is ongoing vascular risk even after diabetes has resolved. Although concentrations of sVCAM-1 and E-selectin are not elevated in type 1 diabetic pregnancies compared to normal, higher levels are seen in those who develop preeclampsia, consistent with the vasculopathy associated with this condition.

## 16

### Effect of albuminuria on the course and outcomes of pregnancy in women with Type 1 diabetes

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**Background and aims:** Of the present work was to evaluate the effect of albuminuria on the course and outcomes of pregnancy in women with Type 1 diabetes (T1DM) and reveal retinopathy progression in these women.

**Materials and methods:** In total 116 women with T1DM were enrolled in the study, they were separated into 3 groups: Gr.1 - 78 (67.3%) women with normal urinary albumin excretion (UAE < 30 mg\24 h), Gr.2–28 (24.1%) women with microalbuminuria (UAE < 300 mg\24 h), Gr.3–10 (8.6%) women with macroalbuminuria (UAE > 300 mg\24 h).

**Results:** The women in Gr.3 were older (29.4 ± 5 yrs.) and had longer diabetes duration (9.2 ± 7yrs.), than in Gr.1 and 2 ($P< 0.05$). At conception good glycemic control was achieved; and it was maintained throughout the pregnancy. We did not register statistically evident difference in HbA1 c (%) between the three groups : Cr.1-7.4 ± 0.3, 6.8 ± 0.4, 6.2 ± 0.2; Gr.2-7.6 ± 05, 6.4 ± 0.3, 6.0 ± 0.4; Gr.3 - 8.1 ± 0.5, 7.1 ± 0.6, 6.4 ± 0.3, (for 1st, 2nd ,3rd trimesters(tr.), respectively). In Gr.2 pre-pregnancy microalbuminuria was detected in 28 women; their number increased to 34 in the 1st tr., and to 38 by term. In Gr.3 macroalbuminuria was observed in 10 women prior to conception, their number increased to 16 by term. During pregnancy all patients experienced gradual increase in daily amount of protein excreted: 1st tr. - 665 ± 425 mg\24 h; 3rd tr. - 1 719 ± 1 156 mg\24 h. Preproliferative retinopathy was found in 5(6.4%) Gr.1, 13(46.4%) Gr.2, and 5(50.0%) Gr.3 patients. Five Gr.3 patients had proliferative retinopathy (50.0%). There was no retinopathy progression after deliveryin in Gr.1 and 2, while in two (20.0%) Gr.3 patients retinopathy progression was observed. Preeclampsia developed in 6.4, 28.5, and 60.0% of women ($P<0.001$); preterm delivery in 12.8, 42.8 and 90.0% ($P<0.001$); mean infants' birth weight was 3 500 ± 410g., 3 300 ± 450g., and 2 850 ± 310g., in Gr.1,2 and 3, respectively. In Gr.2 one (3.5%) and in Gr.3 six (60.0%) newborns had respiratory distress syndrome. In Gr.3 two (20.0%) perinatal deaths were registered.

**Conclusion:** In women with T1DM microalbuminuria at conception may be used as a predictor of pre-eclampsia and pre-term delivery, while macroalbuminuria may predict to retinopathy deterioration, fetal hypotrophy, respiratory distress syndrome and antenatal death. No retinopathy progression was observed, when women with normo- and microalbuminuria were well-controlled throughout the pregnancy.

## 17

**Can measurement of the albumin/creatinine ratio in random urine samples replace 24-hour urine collection in screening for micro- and macroalbuminuria in pregnant woman with Type 1 diabetes mellitus?**

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**Background and aims:** Microalbuminuria is a good predictor for development of pre-eclampsia in pregnant women with type 1 diabetes mellitus. 24-hour urine collection is the traditional method for quantifying the urinary albumin excretion. However, the collection and analysis of these specimens is cumbersome and time consuming for both patient and laboratory and may be associated with collection errors and poor compliance. Thus, there is a need of a more patient friendly alternative. The aim of this study was to determine whether measurement of the albumin/creatinine ratio in random urine samples can replace 24-hour urine collection in screening for micro- and macroalbuminuria in pregnant women with type 1 diabetes mellitus
**Materials and methods:** 119 pregnant women with type 1 diabetes mellitus collected two 24-hour urine samples and two random urine samples on two different days in early pregnancy. The median of the 24-hour urinary albumin excretion in the 2 samples was compared to the median of the albumin/creatinine ratio in the randomly voided specimens. Microalbuminuria was defined as urinary album excretion 30–300 mg/24 h in at least 2 urine samples. Two different lower cut off values of the albumin/creatinine ratio for microalbuminuria, 2.5 mg/mmol and 3.5 mg/mmol, were examined.
**Results:** A positive correlation between the median of the 24-hour urinary albumin excretion and the median of the albumin/creatinine ratio in the random urine samples was found, $R=0.8$ and $P<0.001$. Fifteen of sixteen women with an albumin excretion > 30 mg /24 h had an albumin/creatinine ratio > 2.5 mg/mmol (sensitivity 94%). All 103 women with an albumin excretion < 30 mg /24 h had an albumin/creatinine ratio < 2.5 mg/mmol (specificity 100%). Using the higher cut off for microalbuminuria >3.5 mg /mmol we found that the sensitivity was only 83% while the specificity remained at 100%.
**Conclusion:** Measurement of the albumin/creatinine ratio in 2 random urine samples is a highly specific and sensitive method for screening for microalbumiuria and a good alternative to collecting 24-hour urine samples in pregnant women with type I diabetes mellitus. We recommend that the lower cut off value for microalbuminuria is an albumin/ creatinine ratio = 2.5 mg/mmol in pregnant women.

## 18

**Reduced prevalence of early preterm delivery in women with diabetes Type 1 and microalbuminuria - possible effect of early antihypertensive treatment during pregnancy**

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**Background and aim:** In a cohort of normotensive women with type 1 diabetes and microalbuminuria we have previously documented a very high prevalence of preterm delivery - mainly caused by preeclampsia. The prevalences of preterm delivery before 34 weeks of gestation and preeclampsia were 23% and 42%, respectively. Antihypertensive treatment was mainly initiated in late pregnancy when diastolic blood pressure exceeded 90 mm Hg. Thus the aim of the present study was to study if pregnancy outcome was improved in a new cohort of normotensive pregnant women with type 1 diabetes and microalbuminuria after implementation of a more aggressive antihypertensive treatment strategy.
**Materials and methods:** The old cohort consisted of 26 pregnant women with type 1 diabetes from the period 1995 to 1999 and the new cohort consisted of 20 pregnant women with type 1 diabetes from the period 2000 to 2003. Both groups had microalbuminuria (urinary albumin excretion (UAE) 30–300 mg/24 h in at least 2 out of 3 urine samples) in early pregnancy and attended our clinic before 17 weeks of gestation. Onset of antihypertensive treatment, preeclampsia and preterm delivery, respectively, were recorded similarly in the two cohorts. After April 2000 we recommended onset of antihypertensive treatment with methyldopa in pregnant normotensive women with microalbuminuria if antihypertensive treatment with i.e. ACE-inhibitors was given prior to pregnancy, if proteinuria exceded 3 g/24 h (UAE more than 2 g/24 h), or if blood pressure exceeded 140/90 mm Hg.
**Results:** The old and the new cohorts were comparable with regard to age, duration of diabetes 19 (5) vs. 18 (8) years (mean (SD)), pre-pregnancy BMI 26 (5) vs. 26 (6) kg/m2, early pregnancy UAE 69 (16–278) vs. 74 (30–287) mg/ 24 h (geometric mean and range), blood pressure 121(13)/71 (8) vs. 121 (14)/73 (8)mm Hg and the HbA1 c level during pregnancy (6.7 (0.6) vs. 6.8 (0.5)%), respectively. All had serum creatinine within the normal range. Early antihypertensive treatment in women with microalbuminuria was initiated in 8 (40%) out of 20 women in the new cohort. The presence of preeclampsia was reduced from 11 to 4 women ($p=0.11$), preterm delivery before 34 weeks of gestation from 6 to 0 ($p= 0.02$), preterm delivery before 37 weeks of gestation from 16 to 8 ($p=0.15$). Perinatal mortality occurred in 1 vs. 0 and birth weight was 3124 (767) g vs. 3279 (663) g, respectively.
**Conclusion:** A significant reduction of the prevalence of preterm delivery before 34 weeks of gestation was demonstrated after implementation of antihypertensive treatment with methyldopa in normotensive pregnant women with diabetes type 1 and microalbuminuria.

# OP 4

# Insulin resistance molecular mechanism

## 19

**Increased intramyocellular lipid content in Type 2 diabetes may involve dysregulation of the liver X receptor**

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**Background and aims:** Liver X receptors (LXR) are known as important factors in cholesterol and lipid metabolism, and recently also glucose metabolism. Little is known about the role of LXRs in skeletal muscle. This study investigates the effects of a LXR agonist, T0901317, on lipid and glucose metabolism using myotubes established from type 2 diabetic (T2D) and healthy control subjects.
**Materials and methods:** Fully differentiated human myotubes were exposed to 0.1–1.0 μM T0901317 for 4 days, and then incubated for 4 h either with [$^{14}$C]-glucose, [$^{3}$H]-deoxyglucose or [$^{14}$C]-palmitic acid (PA). RNA was harvested and quantified by RT-PCR.
**Results:** T0901317-treatment increased PA uptake by 50% in control myotubes. Interestingly, the absolute increase was 30% higher in cells from T2D subjects. Further analysis showed that the intracellular level of free PA was increased by 80%, and PA incorporation into phospholipid, diacylglycerol (DAG), triacylglycerol (TAG) and cholesterol ester was increased by 30, 120, 100 and 20%, respectively in control myotubes, whereas T2D-derived myotubes showed an additional 70–80% increase in DAG and TAG formation. T0901317-treatment of control myotubes also increased PA oxidation to $CO_2$ by 22%. This effect on lipid oxidation was absent in T2D cells. Insulin-stimulated glucose transport tended to increase after T0901317-treatment, while glucose oxidation was markedly increased by 65%. No differences were found between T2D and control myotubes. T0901317-treatment increased the mRNA expression of the fatty acid transporter CD36 (2-3-fold), ACS-2 (3-fold), GLUT4 (6-fold), GLUT1 (2-fold), LXRα (8-fold) and PPARγ (3-fold). LXRβ was modestly increased by 50% in control myotubes.
**Conclusion:** This study shows that chronic LXR-activation increases uptake and accumulation of PA to lipids to a higher extent in T2D than control myotubes, which might involve an absent increase in oxidation of PA to $CO_2$ in T2D cells. On the other hand, T0901317-treatment seemingly has a similar positive effect on glucose metabolism for both groups. These findings imply that the increased intramyocellular TAG found in muscle from T2D subjects could be related to dysregulation of the LXR pathway, indirectly leading to insulin resistance and T2D.

## 20

**Regulation of mouse GLUT2 gene expression by glucose in liver**

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**Background and aims:** GLUT2, glucose transporter type 2, is mainly expressed in liver and plays an important role in glucose homeostasis in living organism. Glucose is known to increase GLUT2 mRNA levels and stimulate GLUT2 promoter activity in liver cells. Although intracellular metabolites of glucose may contribute to transcriptional stimulation, molecular mechanism how glucose activates GLUT2 transcription is largely unknown. To understand this pathway, we have searched responsible elements mediating glucose effects in the promoter regions of mouse GLUT2 promoter and would like to find the presence of putative SREBP response element (SRE).
**Materials and methods:** All reagents including cell culture, such as media, fetal bovine serum, antibiotics, and Lipofectamine plus were purchased from Life Technologies and Sigma. Total cellular RNAs were extracted from livers of ICR mouse by using TRIzol reagent method (Invitrogen) and prepared according to manufacturer's protocol. Labeling of each cDNA probe (SREBP-1 c and GLUT2) with [α-$^{32}$P]dCTP was performed by random priming (Amersham International). The serial deletion of constructions was cloned by PCR. Transient transfection was performed with PLUS reagent and Lipofectamine in OPTI-MEM I (Life Technologies) media. Electrophoretic mobility shift assay (EMSA) was tried to single stranded sense oligonucleotide were labeled with [γ-$^{32}$P] ATP using T4 polynucleotide kinase and annealed with unlabeled antisense oligonucleotides. The labeled probe (50,000 cpm) was incubated with nuclear extract from mouse liver for 30 min on ice. The ChIP assay protocol was adapted from methods as described by Real-time PCR was carried out on a LightCycler instrument (Roche Diagnostics GmbH, Germany).
**Results:** Northern blot analysis showed that treatment of insulin or glucose stimulated SREBP-1 c expression. Insulin and glucose were shown to have an additive effect on SREBP-1 c expression. However, GLUT2 expression was not affected by insulin alone. These effects were further confirmed by semi-quantitative real time PCR. Dominant-negative-acting SREBP-1 c inhibited GLUT2 gene expression, whereas constitutively active SREBP-1 c increased GLUT2 level. Transient transfection with serial deletion of the construct revealed that the promoter activity was decreased when −75 region was deleted. DNase I footprinting using GLUT2 promoter fragment (−389/+1) showed 3 protected regions (Site I, II, III). Computer search for the consensus sequences in this region suggested that SRE could be located within Site I. Moreover, enzyme mobility shift assay and mutational analysis of the GLUT2 promoter suggest that stimulatory effect of glucose requires SREBP-1 c. The binding of SREBP-1 to GLUT2 promoter was increased by glucose or glucose/insulin treatment in ChIP assay.
**Conclusion:** These data suggest that the SRE (−84/−76) in GLUT2 promoter may be responsible for SREBP-1 c binding and functional in glucose-mediated GLUT2 gene transcription in hyperglycemic liver. From these findings, it is assumed that glucose mediated activation of GLUT2 gene in liver may occur through SREBP-1 c and can occur in the absence of insulin.
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## 21

**AICAR stimulates adiponectin and inhibits cytokines in human adipose tissue**

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**Background and aims:** AICAR (5-aminoimidazole-4-carboxamide ribonucleo-side) can be used as an experimental tool to activate 5'-AMP-activated protein kinase (AMPK), and has been shown to improve insulin sensitivity. In parallel adiponectin, also seems to activate AMPK and to improve insulin sensitivity. We have investigated the effects of AICAR on the gene expression of adiponectin and on gene expression and release of cytokines in human adipose tissue *in vitro*. In parallel AMPK activity was investigated.
**Materials and methods:** Subcutaneous adipose tissue was obtained from 10 healthy subjects (BMI 25.6 ± 1.2 kg/m$^2$). Adiponectin and cytokine mRNA was determined by real-time RT-PCR. Cytokine concentration was determined by ELISA.
**Results:** AICAR stimulated AMPK α1 activity 3–4 fold ($p<0.001$), and dose-dependently increased adiponectin mRNA levels with significant stimulation (2–4 fold increase) at AICAR concentrations of 0.5–2 mM ($p < 0.05$). The increase was present after six hours of incubation, and levelled of after 24 hours approaching the control. The release of TNF-α and IL-6 was decreased by AICAR ($p<0.05$).
**Conclusion:** In conclusion, AICAR stimulated adipose tissue AMPK α1 activity and adiponectin gene expression, while attenuating the release of TNF-α and IL-6. Reduced concentrations of these cytokines and increased levels of adiponectin might play a role for the insulin sensitizing effects of AICAR.

## 22

**Nuclear factor κB-activation is central for the decrease of glucose-6-phosphatase gene expression by tumor necrosis factor, but not by insulin**

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**Background and aims:** The key gluconeogenic enzyme glucose-6-phosphatase (G6Pase) has an important function in the control of hepatic glu-

cose production and the expression of its gene is increased in diabetes. This is probably caused by a deregulation of signalling events controlling G6Pase gene transcription. In order to characterize these pathways, we examined the role of the nuclear factor κB (NFκB)-pathway in the regulation of G6Pase gene transcription by insulin and tumor necrosis factor (TNF).
**Materials and methods:** G6Pase expression was determined in hepatoma cells by Northern Blot analysis. Wild type inhibitor of κB (IκB) and a dominant-negative mutant of IκB were overexpressed using adenoviral-mediated gene transfer. NFκB-activation was assessed by an ELISA. The effect of transiently overexpressed NFκB was studied using G6Pase promoter reporter gene assays.
**Results:** TNF and insulin decreased G6Pase mRNA levels by 45 ± 8% and 80 ± 10% (S.E.M. n=3), respectively. TNF stimulated NFκB activation approximately 4.4 fold, whereas insulin was without effect. TNF and insulin decreased G6Pase expression by 51 + 9% and 85 + 9% (S.E.M., n=3), respectively in cells overexpressing wildtype IκB. The adenoviral overexpression of a dominant negative mutant of IκB completely prevented both the activation of endogenous NFκB and the suppression of G6Pase expression by TNF. However, insulin was still able to decrease G6Pase gene expression by 88 ± 9% (S.E.M., n=3) in cells overexpressing dominant negative IκB. TNF and the transient overexpression of NFκB decreased G6Pase promoter activity concentration-dependently by up to 48 ± 8% and 53 ± 10% (S.E.M. n=3), respectively. Although two binding sites for NFκB were identified within the G6Pase promoter, neither of these sites, nor the insulin response unit or binding sites for Sp proteins, was necessary for the regulation of G6Pase promoter activity by TNF.
**Conclusion:** The data indicate that the activation of NFκB is sufficient to suppress G6Pase gene expression and is required for the regulation by TNF, but not by insulin. We propose that NFκB does not act by binding directly to the G6Pase promoter.
*Supported by: the DFG and FEBS*

## 23

### COX-2 inhibitors induce up-regulation of glucose transport in L6 myotubes by activating PKCδ

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**Background and aims:** Several case reports on adverse effects of cyclooxygenase 2 (COX-2) inhibitors suggest that an overdose consumption or their combination therapy with antihyperglycemic drugs may induce hypoglycemic episodes in man. The aim of this study was to investigate whether COX-2 inhibitors (nimesulide, niflumic acid and rofecoxib) exert this effect by upregulating the glucose transport system in skeletal muscle cells.
**Materials and methods:** The effect of a COX-2 inhibitor on blood glucose level was studied in male diabetic GK rats. L6 myotubes were used to study the effects of COX-2 inhibitors on the glucose transport capacity, GLUT-4 expression and cellular distribution. Insulin or insulin-like effects were investigated by following activation of key elements in transduction mechanisms known to stimulate glucose transport in skeletal muscles.
**Results:** We found that the COX-2 inhibitor, nimesulide, augmented the blood glucose-reducing effect of gliclazide in diabetic LPS-treated GK rats, in which COX-2 expression was induced in skeletal muscles. Therapeutic doses of nimesulide had no effect on blood glucose in control rats that did not express COX-2. Cultured L6 myotubes express COX-2 and its cellular content was higher under hyperglycemic conditions than under normoglycemic conditions. All three inhibitors augmented the rate of glucose transport in myotubes maintained under hyperglycemic conditions in a dose- and time-dependent manner by increasing total cell content of GLUT-4 mRNA and protein and its plasma membrane abundance. These effects were insulin-independent and obtained also in the presence of the PI3-kinase inhibitor LY294,002. Unlike insulin, the inhibitors did not induce site-specific phosphorylation of IRS-1, Akt/PKB, GSK3α/β, αAMPK, c-Cbl, JNK1/2, ERK1/2 or p38. However, like insulin, they activated mTOR and induced prominent p70S6K and 4EBP1 specific phosphorylation. Yet, this effect was not related to their glucose transport augmenting activity, since it was not altered in the presence of rapamycin, which effectively inhibited mTOR-dependent p70S6K and 4EBP1 phosphorylation in the absence or presence of the inhibitors. Moreover, the inhibitors induced tyrosine phosphorylation of PKC δ. Rottlerin, at a concentration known to inhibit PKC δ activation, abolished both COX-2 inhibitor- and insulin-dependent phosphorylation of PKCδ, as well as their glucose transport augmenting activities. The inhibitors failed to increase glucose transport in L6 myotubes, which expressed a dominant-negative form of PKCδ.
**Conclusion:** COX-2, whose expression is increased under hyperglycemic conditions in L6 myotubes or in intact skeletal muscles under inflammatory conditions may mediate the downregulation of glucose transport seem under hyperglycemic conditions. Since insulin-dependent PKCδ activation has been shown to increase glucose transport in skeletal muscle cells, we suggest that COX-2 inhibitors mimic this effect. The mechanism by which COX-2 inhibitors induce PKCδ activation remains to be investigated. However, these COX-2 inhibitors-induced activities may be related to their potential hypoglycemic effect in man.
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## 24

### Platelets from obese subjects are resistant to both cyclic GMP and cyclic AMP, the final mediators of platelet anti-aggregation: role of insulin resistance

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**Background and aims**: Subjects with central obesity – a classical condition of insulin resistance – exhibit platelet hyperactivation, which is involved in the atherosclerotic process and can therefore account for their increased risk of cardiovascular morbidity and mortality. In previous studies, we demonstrated in human obesity a resistance to the anti-aggregating effects of substances which activate the nitric oxide (NO)/guanylate cyclase/cyclicGMP (cGMP) pathway -such as insulin and organic nitrates – and of substances which activate the adenylate cyclase/cyclicAMP (cAMP) pathway – such as prostacyclin and adenosine. Aim of the present study is to evaluate whether platelets from obese subjects are resistant to the actions of the two final effectors of anti-aggregation, i. e. cGMP and cAMP.
**Material and methods:** Platelet-rich plasma (PRP) was obtained from 8 obese subjects (4 M and 4 F, age: 35.4 ± 2.1 years, BMI: 33.2 ± 1.6, waist circumference: 109.2 ± 3.1 cm, HOMA IR index: 4.8 ± 0.6) and from 6 control subjects (3 M and 3 F, age:34.7 ± 1.9 years, BMI: 21.5 ± 0.4, waist circumference: 80.5 ± 2.6 cm, HOMA IR index: 1.3 ± 0.1). All subjects showed a normal glucose tolerance. In PRP samples, platelet response to 4 μmol/l ADP, evaluated by the Born's method, was measured in the presence of: i) the cell permeable cGMP analog pCPT-cGMP, a selective activator of the cGMP-dependent protein kinase (PKG), lacking of significant inhibitory effects on phosphodiesterases and therefore unable to increase cAMP concentrations (10–500 μmol/l; incubation times: 3-20 min) and ii) the cell permeable cAMP analog pCPT-cAMP, a selective activator of cAMP-dependent protein kinase (PKA) (10–500 μmol/l; incubation times: 3-20 min). IC-50 (i.e. the minimal concentration of inhibitors necessary to reduce platelet response to ADP by half) was determined.
**Results:** The anti-aggregating effect of both cyclic nucleotides was smaller in obese subjects, who presented a lower decrease of platelet response to ADP with each cGMP or cAMP concentration at each incubation time; in particular, pCTP-cGMP IC-50 was: in control subjects, 111 ± 52, 54 ± 30 and 17 ± 8 μmol/l at 3, 10 and 20 min, respectively; in obese subjects, not detectable at 3 and 10 min since a platelet aggregation reduction greater than 50% of basal was not reached, and 172 ± 40 μmol/l at 20 min (p=0.004 vs controls); pCTP-cAMP IC-50 was: in control subjects, 94 ± 34, 24 ± 7 and 5 ± 1 μmol/l at 3, 10 and 20 min, respectively; in obese subjects, not detectable at 3 min since a platelet aggregation reduction greater than 50% of basal was not reached, 252 ± 41 μmol/l at 10 min (p=0.0001 vs controls) and 172 ± 40 μmol/l at 20 min (p=0.007 vs controls). Both pCTP-cGMP IC-50 and pCTP-cAMP IC-50 values obtained with a 20-min incubation correlated with the insulin resistance index HOMA IR (r=0.922, p=0.0001 and r=0.941, p=0.0001, respectively) and with waist circumference (r=0.766, p=0.001 and r=0.813, p=0.0001, respectively).
**Conclusions:** The resistance to both cGMP and cAMP, final mediators of platelet anti-aggregation, in platelets from patients affected by central obesity explain why they are resistant to different anti-aggregating substances (such as insulin, nitric oxide, prostacyclin and adenosine), and is likely the basic defect accounting for the platelet hyperactivation of human obesity. Insulin resistance plays a major role in this phenomenon.
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# OP 5

# Lipids in diabetes: from basics to clinical complications

## 25

**The expression of intestinal ATP binding cassette proteins G5 and G8, the regulators of cholesterol homeostasis, is reduced in Type 2 diabetic patients**

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**Background and aims:** Atherosclerosis is a major complication of type 2 diabetes. The dysregulation of cholesterol metabolism may play a central role. We have shown that intestinal cholesterol synthesis is increased in experimental diabetes and inhibition of cholesterol synthesis has been shown to be an effective treatment in patients with diabetes. Cholesterol homeostasis has been shown to be regulated by the ATP binding cassette (ABC) transporters G5 and G8. In the intestine they work in tandum to facilitate cholesterol excretion and in the liver they regulate the cholesterol content of bile. A recent study in diabetic animals found ABC G5 mRNA expression and protein to be reduced and cholesterol absorption increased. The present study examines intestinal ABCG5 and G8 mRNA levels in type 2 diabetic patients.
**Materials and methods:** Diabetic patients and control subjects undergoing routine gastroscopy had duodenal biopsies taken, ethics committee approval having been obtained. Patients found to have abnormal gastric or intestinal mucosa were excluded. ABC G5 and G8 mRNA expression was measured by 2 step RT-PCR using an ABI Prism 7000 sequence detector. Primer and probes were obtained from Applied Biosystems using the "assay on demand system". Results were expressed in arbitary units using GAPDH as housekeeping gene. Fasting plasma cholesterol and indices of diabetic control were determined by routine laboratory methods.
**Results:** Nine diabetic patients and 14 control subjects were included in the study. There was no significant difference in age or BMI between diabetic and control subjects. Mean HbA1 in the diabetic patients was 6.7 ± 1%. Plasma cholesterol was similar in the diabetic patients and in the control subjects (4.3 ± 0.9 vs 5.1 ± 1.2 mmol/l). There was a positive correlation between ABCG5 and ABCG8 expression in the whole group ($r=0.51$, $p<0.002$). The relative gene expression of ABCG5 was reduced by 40% in the diabetic patients compared to control subjects ($p<0.005$) and ABC G8 was reduced by 33% in diabetic patients ($p<0.03$). There was a negative correlation between both ABC G5 and G8 and plasma cholesterol in the diabetic patients ($r=-0.61$ $p<0.05$ and $r=-0.57$, $p<0.055$) while in control subjects there was no correlation between cholesterol and G5 or G8.
**Conclusion:** The negative correlation between ABCG5 and G8 and cholesterol in the diabetic subjects suggests that up-regulation of these transporter genes might be particularly useful in the treatment of diabetic dyslipidaemia.

## 26

**Polymorphisms of ABCA1 transporter gene and HDL-C in the D.E.S.I.R cohort**

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**Background and aims:** The ATP Binding Cassette transporter, ABCA1, plays a key role in the reverse cholesterol transport. Identification of mutations in the ABCA1 gene in patients with Tangier Disease (HDL-C deficiency) suggested that single nucleotid polymorphisms (SNP) in the ABCA1 gene could determine HDL-C and apolipoprotein-A1 (apoA1) levels. Our aim was to assess the contribution of three SNPs, C69T, G378C, and G1051A on lipid levels in the D.E.S.I.R. cohort (Epidemiologic Data on the Insulin Resistance Syndrome), a representative sample of the France general population.
**Materials and methods:** There were 2576 men and 2636 women aged 30–65 years, who volunteered in D.E.S.I.R. study in Health Examination centers in the western central part of France. Clinical, anthropometric and biologic data were been collected at baseline.The SNP were genotyped using PCR-molecular Beacons technique. Associations of genotypes with lipid phenotypes were tested by ANOVA or ANCOVA. Haplotypes and linkage desequilibrum between SNPs were estimated using the Estimating Haplotype program.
**Results:** Allele frequencies (%) in D.E.S.I.R. were 35, 13 and 28 for C69T, G378C and G1051A variants, respectively. The genotype distributions were in Hardy-Weinberg equilibrium with allele frequencies similar to those reported in other Caucasian populations.
The C69T SNP was associated with HDL-C and apoAI concentrations depending on sex and corpulence. In normal weight male (BMI < 25 kg/m²), TT subjects (n = 137) had higher HDL-C, and apo-AI (1.65 ± 0.29 mmol/l and 1.64 ± 0.28 g/l respectively) than in 69 C allele carriers (n=1071 ; 1.56 ± 0.32 mmol/l and 1.59 ± 0.28 g/l respectively) ($p = 0.009$ and $p = 0.049$ for HDL-C and apo-AI, respectively). For G378C, in the total population, the 378 C allele (n = 1295) was associated with lower HDL-C (1.60 ± 0.37 mmol/l in C+ vs 1.64 ± 0.32 mmol/l in GG ; $p = 0.002$) and apo-A1 concentrations, (1.63 ± 0.25 g/l in C+ vs 1.65 ± 0.2 g/l in GG; $p=0.003$) respectively. This effect was linked to sex and corpulence. Overweight and obese male carriers of 378 C variant (n = 969) had significantly lower HDL-C (1.35 ± 0.33 mmol/l in C+ vs 1.42 ± 0.32 mmol/l in GG ; $p = 0.003$) and apo-AI (1.52 ± 0.22 g/l in C+ vs 1.55 ± 0.22 g/l in GG ; $p=0.027$).
The G1051A SNP influences HDL-C in interaction with Body Mass Index ($p<0,001$). In normal weight population (n =2959), the 1051 A allele (n = 1421) was associated with higher HDL-C concentration (1.77 ± 0,32 mmol/l in A+ vs 1.71 ± 0.31 mmol/l in GG ; $p=0,026$). In the overweight and obese population (n = 2148), this variant (n=1047) was associated with a drop in HDL-C concentration (1.48 ± 0,33 mmol/l in A+ vs 1.53 ± 0.34 mmol/l in GG ; $p=0,002$). Haplotypic analysis didn't show any linkage desequilibrum between these SNPs. Haplotypic combinations of these SNPs were not significatively associated with HDL-C concentration.
**Conclusion:** This study suggests that in the D.E.S.I.R cohort, polymorphisms of the ABCA1 gene modulat the HDL-C and apoAI concentrations, in interaction with sex and corpulence. Therefore, they might influence the cardiovascular risk in the general population.

## 27

**Influence of G378C and R219K polymorphisms of ABCA1 transporter gene on lipids and vascular diseases in Type 2 diabetic subjects**

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**Background and aims:** The ATP Binding Cassette A1 transporter (ABCA1) plays a key role in high density lipoprotein (HDL) metabolism. It mediates the efflux of phospholipids and free cholesterol from peripheral cells to apolipoprotein A-I, reversing foam cell formation. Mutations in *ABCA1* gene cause Tangier disease and familial hypoalphalipoproteinemias, two disorders with low plasma HDL cholesterol (HDL-C). Our aim was to study the associations of two single nucleotide polymorphisms (SNPs) of *ABCA1* (G378C, R219K) with serum lipids and micro and macrovascular diseases in a large group of type 2 diabetic (T2D) subjects.
**Materials and methods:** We studied 3757 T2D subjects: 3150 French participants to the Non-Insulin-Dependent Diabetes, Hypertension, Microalbuminuria, Cardiovascular Events and Ramipril (DIABHYCAR) Study, and 607 control subjects of the same age, sex ratio and geographical origin with normoalbuminuria. The SNPs were genotyped by PCR amplification coupled to a fluorescent specific probe hybridization (PCR-Molecular Beacons). Effects of genotypes on lipids were tested by analysis of variance and covariance, and on vascular diseases by logistic regression. Haplotypes and linkage disequilibrium between SNPs were estimated with the Estimating Haplotype program.
**Results:** In total, the CC homozygotes for the G378C polymorphism (n = 70) had lower HDL-C (1.24 ± 0.04 mmol/l) than 378G allele carriers (1.34 ± 0.01 mmol/l, $p = 0.023$). G378C was not associated with macrovascular diseases. However, the 378C allele frequency was decreased in patients with diabetic retinopathy (n = 151, 17%) when compared to subjects without (25%, odds ratio [OR]: 0.620; 95% confidence interval [CI]: 0.404–0.952; $p = 0.028$). The R219K polymorphism was not related to lipids in the total population. Nevertheless, in the overweight group (body mass index >= 25 kg/m², n = 255), the 219KK homozygotes had lower total cholesterol (5.66 ± 1.17 mmol/l) than the others (5.85 ± 1.12 mmol/l, $p = 0.009$). The 219K allele was less frequent in participants with cardiovascular diseases (n = 860, 46%) than in those without (50%, OR: 0.856; 95% CI: 0.735–0.998; $p = 0.047$). These two polymorphisms were not in linkage disequilibrium ($Chi^2 = 7.629$, $p = 0.106$). The haplotype analyses on retinopathy and macrovascular diseases did not show any additional results.
**Conclusion:** This study shows polymorphisms in ABCA1 gene are associated with lipid levels and with some micro and macrovascular complications in T2D.

## 28

**Poorer glycemic control is associated with dyslipidemia in Type 2 diabetes**

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**Background and aims:** Currently, there is limited information on the prevalence of diabetic dyslipidemia or its relationship to glycemic control. The objectives of this study were to describe the subgroups of lipid abnormalities in type 2 diabetes as well as the cross-sectional relationships between these subgroups and other patient characteristics, including glycemic control.
**Materials and methods:** The study population included all 11,935 members of Kaiser Permanente Northwest, a 450,000 member HMO, who had type 2 diabetes diagnosed prior to 2001, at least one HbA1 c measurement in both 2001 and 2002, and a fasting lipid panel in either 2001 or 2002. We then used the ADA criteria for categorizing high, borderline and low cardiovascular risk based on LDL-C, HDL-C, and triglycerides.
**Results:** Nearly all patients (98.4%) had at least one borderline-risk lipid abnormality; 84.5% had two or more. More importantly, 70% of all patients had at least one high-risk lipid abnormality. Low HDL-C was the most common high-risk abnormality (59.8% of subjects), followed by high LDL-C (17.9%). HbA1 c levels increased steadily as the number of lipid abnormalities increased. Younger subjects and women were much more likely to have multiple lipid abnormalities. In addition, subjects in each high risk lipid category were in the poorest glycemic control, compared to those in the low and borderline risk categories. This relationship was most dramatic across triglyceride risk categories.

Number of High Cardiovascular Risk Lipoprotein Abnormalities

| | None | One | Two | All Three | Total |
|---|---|---|---|---|---|
| Number of Subjects | 3,668 | 6,294 | 1,898 | 75 | 11,938 |
| Mean Age | 65.8 | 63.7 | 60.3 | 58.7 | 63.8 |
| Mean Duration of Diabetes (years) | 6.4 | 6.1 | 5.8 | 6.1 | 6.1 |
| Percent Female | 42.9% | 48.8% | 56.2% | 72.0% | 48.3% |
| Mean HbA1 c | 7.3% | 7.5% | 7.9% | 8.8% | 7.5% |
| Mean LDL-C | 100 | 105 | 120 | 154 | 106 |
| Mean HDL-C | 54 | 40 | 37 | 39 | 44 |
| Mean Triglycerides | 150 | 210 | 381 | 509 | 221 |
| Mean Blood Pressure | 137/77 | 137/78 | 138/80 | 137/80 | 137/78 |

**Conclusion:** Our study is the first to describe combinations of lipid abnormalities in a representative population of persons with type 2 diabetes. Persons with diabetes are at high risk of cardiovascular disease and mortality. The association between glycemic control and lipid levels emphasizes that cardiovascular health in diabetes requires a multi-faceted approach, especially in younger patients and women.

## 29

**Prospective impact of triglycerides versus cholesterol in diabetic coronary patients**

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**Background:** Diabetes mellitus type 2 (DM2) is accompanied by a typical pattern of dyslipidemia (hypertriglyceridemia with low HDL and elevated total cholesterol) and is strongly atherogenic. It is not clear which if any of the dyslipidemic components predicts atherosclerotic disease because prospective data in patients with both diabetes and coronary artery disease are scarce.
**Methods:** We enrolled 756 consecutive patients undergoing coronary angiography. Patients with diabetes type 1 (n = 6) were excluded from the analyses. Lipid values were estimated in fasting serum samples; LDL was measured directly with QuantolipLDL (Roche, Switzerland). The incidence of vascular end points was recorded during a follow-up period of 2.3 ± 0.4 years. Factorial analysis was applied to extract factors from the lipid profile of our patients.
**Results:** Factorial analysis revealed two factors in the lipid profiles of our patients: Total cholesterol, LDL cholesterol, and apoB loaded on factor 1, and triglycerides, HDL cholesterol, and ApoA1 loaded on factor 2. Coronary patients with DM2 (n = 164) had significantly higher triglycerides (203 ± 139 vs. 154 ± 92 mg/dl; p <0.001) and lower HDL cholesterol (44 ± 14 vs. 50 ± 14 mg/dl; p <0.001) than non-diabetic coronary patients. Interestingly, LDL cholesterol was significantly lower in patients with DM2 (121 ± 36 vs. 134 ± 35; p <0.001). ApoB was similar in diabetic and nondiabetic patients; thus the LDL cholesterol /ApoB ratio was significantly lower in diabetic patients (p <0.001) as was the LDL peak particle diameter (257 ± 7 vs. 259 ± 7; p = 0.039). The triglyceride-driven factor 2, but not the cholesterol-driven factor 1 was associated with the diabetic status. Prospectively, after adjustment for age and gender, the triglyceride-driven factor 2 was significantly predictive for vascular events in the total study cohort (p = 0.002) and among patients with DM2 (p = 0.036), but not in the nondiabetic subgroup. The cholesterol-driven factor 1 was not associated with vascular events in either study subgroup nor in the total cohort.
**Conclusions:** In addition to the classical pattern of diabetic dyslipidemia coronary patients with DM2 exhibit low serum levels of true LDL cholesterol. Among diabetic coronary patients the triglyceride factor but not the cholesterol factor proved predictive for vascular events.

## 30

**Effect of Pioglitazone on HDL cholesterol levels in comparison to metformin and gliclazide according to IDF-categories for vascular risk**

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**Background and aims:** Lipid abnormalities such as low HDL cholesterol are well known indicators of cardiovascular risk and more prevalent in type 2 diabetic patients who are at risk of increased cardiovascular morbidity and mortality compared to non-diabetic patients From epidemiological studies it is known that a 1% increase in HDL cholesterol is associated with about 2–3% reduction in the risk of coronary heart disease. Following the guidelines according to the European Diabetes Policy Group (IDF;1999), which collates HDL cholesterol levels to the risk for vascular complications, a low risk category is defined by a HDL cholesterol level of >1,2 mmol/l, an increased risk exists at a level of 1,0–1,2 mmol/l, whereas the vascular risk is high at < 1,0 mmol/l.
**Materials and methods:** HDL cholesterol levels were recorded in four large double-blind, randomised active comparator controlled European trials in over 3700 patients. The trials compared treatment with pioglitazone (as monotherapy or combination therapy) with treatment with either gliclazide or metformin (as monotherapy or combination therapy) for one year.
**Results:** The patients recruited into the trials had an average age of 57 years and baseline $HbA_{1c}$ of 8,7%. Mean BMI was 31. In both monotherapy and combination therapy trials pioglitazone increased HDL cholesterol levels consistently from baseline values and caused larger elevations of HDL cholesterol than gliclazide or metformin. This difference cannot be explained just by the improvement in glycaemic control, as this was similar between the treatments. Increases in HDL cholesterol levels with pioglitazone were about 15%-20% in all four studies. These absolute values correspond to remarkable changes in risk categories for vascular complications, as over 40% of pioglitazone treated patients lowered their cardiovascular risk by at least one risk category.
**Conclusion:** Treatment with pioglitazone leads to an increase of HDL cholesterol values. This translates to different results in risk categories for vascular complications.

Effect of PIO vs. Comparators on HDL Cholesterol according to IDF-Categories for Vascular Risk

| Study | Percentage of patients with change of at least one risk category | | | |
|---|---|---|---|---|
| | HDL-C, Risk reduction | | HDL-C, Risk increase | |
| | PIO | Comparator | PIO | Comparator |
| EC 404 (vs. Metformin mono) | 43,7 | 25,6 | 4,3 | 7 |
| EC 405 (vs. Gliclazide mono) | 46,7 | 23,5 | 2,4 | 9,3 |
| EC 409 (vs. Metformin combo) | 42,5 | 29,7 | 4,4 | 7,7 |
| EC 410 (vs. Gliclazide combo) | 41 | 17,4 | 2,3 | 14,5 |

# OP 6

# „Classical" oral agents

## 31

**Beta-cell response to metformin-glibenclamide combination tablets in Type 2 diabetes: results of a multicentre, randomised, double-blind trial**

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**Background and aims:** This study explored the β-cell response to metformin and glibenclamide* administered as a single-tablet combination (Glucovance®), or as monotherapies. The primary objective was to assess the percent change from baseline in the 2nd-phase insulin response after 20 weeks of treatment with metformin-glibenclamide combination tablets, glibenclamide or metformin, in patients inadequately controlled by diet and exercise.
**Materials and methods:** Eligible patients (aged 20–75 years, with established type 2 diabetes of <5 years duration and $HbA_{1C}$ >6.7% and <9.5% on diet and exercise) were randomised to metformin-glibenclamide combination tablets or either monotherapy for 20 weeks. Dosages were titrated to glycaemic targets. At baseline, patients underwent a 5 h oral glucose tolerance test (OGTT) with dual-stable isotopic glucose tracers. After an overnight insulin infusion procedure, patients underwent a hyperglycaemic clamp (180 min at 190 mg/dL [10.6 mmol/L]). Both studies were repeated at the final visit. Insulin values were log-transformed. Point estimates and 95% confidence intervals for the geometric mean percent changes in 1st- and 2nd-phase insulin responses were calculated using an ANCOVA model with terms for age and BMI at baseline.
**Results:** Fifty patients were randomised and 45 completed the study. All 3 treatment groups showed improvements in indices of β-cell function associated with a similar final mean $HbA_{1C}$ (7.0–7.4%). An additional improvement in 1st- and 2nd-phase insulin response was apparent in the metformin-glibenclamide combination tablet group (Table). Effects on the insulin sensitivity index (ISI) for combination tablets were intermediate between metformin and glibenclamide. Final mean doses of metformin and glibenclamide in the combination group were approximately 50% lower vs. the monotherapy groups. Combination tablets were well tolerated.
**Conclusions:** In the current trial, Glucovance® treatment resulted in improvements in β-cell function after 20 weeks. This may be a result of earlier absorption of glibenclamide from the combination tablets compared with glibenclamide alone.
*Glyburide in the USA

| Treatment Group | Metformin-glibenclamide combination tablets | Metformin monotherapy | Glibenclamide monotherapy |
|---|---|---|---|
| Mean Age, y (SD) | 49 (12) | 48 (9) | 51 (10) |
| Mean baseline BMI, kg/m² (SD) | 33 (5) | 33 (6) | 36 (4) |
| Mean duration of diabetes, y (SD) | 2.6 (1.3) | 2.7 (2.2) | 2.4 (1.6) |
| Mean baseline $HbA_{1C}$, % (SE) | 8.0 (0.4) | 7.66 (0.27) | 7.81 (0.35) |
| Mean final $HbA_{1C}$, % (SE) | 7.0 (0.2) | 7.4 (0.3) | 7.1 (0.2) |
| Mean Δ $HbA_{1C}$, % (SE) | −0.95 (0.34) | −0.24 (0.34) | −0.7 (0.29) |
| % change 1st-phase insulin[a] | 35% (13, 61) | 15% (−5, 40) | 19% (−2, 44) |
| % change 2nd-phase insulin[a] | 93% (56, 138) | 36% (9, 70) | 46% (15, 84) |
| Mean Δ ISI (pmol/min/kg/pM) | −4 | 11 | −9 |
| Mean final dose (mg) | 708/3.5 mg | 1500 mg | 6.6 mg |
| Hypoglycaemia, n (%) | 2 (11%) | 0 | 5 (29%) |
| Gastrointestinal events, n (%) | 3 (17%) | 8 (53%) | 4 (23%) |

[a]Adjusted geometric mean % change in β-cell indices (95% C.I.).
See text for abbreviations.

*Supported by: Bristol-Myers Squibb*

## 32

**Metformin prevents glucose-induced PKC beta2 activation in human umbilical endothelial cells**

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**Background and aims:** Activation of protein kinase C (PKC) and in particular of the beta2 isoform (PKC beta2), contributes to the development of endothelial dysfunction in diabetes. The specific inhibition of this kinase is in the experimental phase. It is unknown whether insulin sensitizers, such as metformin (MET), influence PKC beta2 activity. We have consequently decided to evaluate: 1) whether hyperglycaemia induces PKC beta 2 activation in isolated human endothelial cells (HUVEC); 2) whether metformin prevents glucose-induced PKC beta 2 translocation from the cytosol to the cell membrane; and 3) the mechanism or mechanisms through which this may occur.
**Materials and methods:** We studied: a) PKC beta 2 translocation in HUVEC, transfected with a PKC beta 2-Green Fluorescent Protein (GFP) chimera via adenovirus, through an epifluorescence microscope system; and b) radical oxygen species (ROS) synthesis using fluorescent probe Tempo-9-AC. The cells were cultured in normal glucose (5 mM) and moderate hyperglycaemic (10 mM) conditions for 48 hours.
**Results:** Under these conditions no translocation of PKC beta 2 was observed, calculated as the difference in the fluorescence ratio of plasma membrane:cytosol of PKC beta 2-GFP chimera before and after treatment. The acute addition of H2O2 (500 μM) induced rapid PKC beta 2 translocation in either condition and could be observed within 10 minutes of H2O2 addition, with an initial fluorescence ratio of 0.01 ± 0.02 that increased to 0.4 ± 0.01 after H2O2 addition. If the cells were treated with hyperglycaemic stimuli (25 mM glucose) for twenty minutes the translocation of PKC beta 2 was observed only in cells that had been cultured with 10 mM glucose. Pretreatment with MET (20 μM, 48 hrs) prevented both hyperglycaemia- and hydrogen peroxide- induced PKC beta 2 translocation. Although a significantly increased ROS synthesis was observed in cells cultured in 10 mM of glucose when compared to normoglycaemic conditions, this glucose-induced effect was abolished by MET.
**Conclusion:** In conclusion our data demonstrate that hyperglycaemia induces activation of PKC beta 2 via oxidative stress, but only in cells chronically exposed to hyperglycaemia; MET is capable of preventing PKC beta 2 activation via a direct antioxidant effect. Our results confirm that MET has beneficial vascular effects that are independent of its glucose-lowering action.

## 33

**Efficacy of orlistat in overweight and obese patients with metabolic syndrome and its effects on the risk of coronary heart disease**

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**Background and aims:** Metabolic syndrome (MS) is a cluster of abnormalities that commonly occur in overweight and obese patients that increases their risk of cardiovascular (CV) disease and type 2 diabetes (T2D). The aim was to assess the relationship between excess weight and the clustering of risk factors, and to examine the effect of orlistat (ORL) in patients with MS.
**Materials and methods:** Data were pooled from 5 randomised, double-blind, placebo (PLA)-controlled trials of ORL 120 mg tid in overweight and obese patients (body mass index ≥25 kg/m²). All patients were prescribed a mildly calorie-reduced diet. Patients with ≥3 of the following National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III criteria were assessed as having MS: abdominal obesity (waist circumference >102 cm men, >88 cm women), high triglycerides (≥1.7 mmol/L), low HDL-cholesterol (<1.0 mmol/L men, <1.3 mmol/L women), hypertension (≥130/85 mm Hg) and high fasting glucose (≥6.1 mmol/L). For efficacy assessments, patients were analysed using the least squares mean (LSM) change from baseline to end of treatment between ORL and PLA.
**Results:** Of the 2673 patients in these trials, more than half (56.2%) had 3, 4 and 5 MS criteria at baseline (ORL n=766; PLA n=737), according to NCEP ATP III criteria. The condition was slightly more common in men (60.2%) than women (54.2%). Patients with MS were approximately 7–8 kg heavier than those without at baseline. At study end, among those with 3 MS criteria at baseline, a significantly greater number of ORL-treated patients (60.9%; 249/409) than PLA recipients (51.7%; 195/377) did not have MS at study end (p=0.0166). Patients treated with ORL achieved a 2.5-fold greater

weight loss than PLA-treated patients (LSM: -5.53 *vs* -2.17 kg; p<0.0001). Importantly, ORL-treated patients with MS demonstrated significant improvements in metabolic abnormalities (abdominal adiposity, hypertension, elevated fasting glucose) compared with PLA recipients by study end (table). A 2-fold greater reduction in waist circumference was achieved by patients treated with ORL than with PLA. In addition, there was a significant difference in the 10-year coronary risk for heart disease between ORL and PLA treatment groups at study end (LSM: -3.62 *vs* -2.09; p=0.0253).
**Conclusion:** Treatment with ORL plus diet led to significantly greater improvements in all metabolic abnormalities in overweight and obese patients with MS compared with PLA plus diet. Importantly, ORL significantly reduced the 10-year coronary heart disease risk in patients with MS.

| Change from baseline | ORL | PLA | p-value |
|---|---|---|---|
| Waist circumference (cm) | -6.26 | -3.15 | <0.0001 |
| $HbA_{1c}$ (%) | -0.21 | +0.01 | <0.0001 |
| Fasting glucose (mmol/L) | -0.73 | -0.19 | <0.0001 |
| Systolic blood pressure (mmHg) | -7.60 | -5.07 | 0.0017 |
| Diastolic blood pressure (mmHg) | -5.68 | -4.30 | 0.0044 |

*Supported by: F. Hoffmann-La Roche*

## 34

**Repaglinide-metformin combination in the treatment of Type 2 diabetes: Comparison between three different combination regimens**
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on behalf of Italian Repaglinide Study Group.

**Background and aims:** Type 2 diabetes is a heterogeneous disorder due to interaction of impaired early insulin secretion and peripheral insulin resistance. Starting from this point of view, the combination between two drugs able to restore respectively early insulin secretion and insulin sensitivity, such as Repaglinide and Metformin, could be a good therapeutical option, fitting with the pathophysiological aspects of Type 2 diabetes. The aim of present study was to compare the efficacy and safety of Repaglinide in combination with Metformin in type 2 subjects not well controlled with sulphanylureas or metformin in monotherapy. In order to set the best combination between Repaglinide and Metformin, we compared three different regimens: Repaglinide 1 mg/meal + Metformin 1500 mg dinnertime (Group 1); Repaglinide 1 mg/meal + Metformin 1000 mg dinnertime (Group 2); Repaglinide 1 mg/meal + Metformin 500 mg/meal (Group 3). Metformin dose was unmodified through the study instead of Repaglinide whose dose could be increased at 2 mg/meal in case of FPG>140 mg/dl after first two weeks from randomization.
**Materials and methods:** A total of 102 Type 2 diabetic subjects not well controlled in OHA monotherapy were randomized. Three groups were well balanced at baseline for sample size (34, 33 and 35 respectively in Group 1, 2 and 3), age (56.2, 54.3 and 57.3 years respectively in Group 1, 2 and 3), duration of disease (6.2, 6.8 and 6,7 years respectively in Group 1, 2 and 3), BMI (29.5, 29.4, 28.9 $Kg/m^2$ respectively in Group 1, 2 and 3) and HbA1 c (8.5, 8.2 and 8.4% respectively in Group 1, 2 and 3). 90 subjects ended the 16 weeks study treatment period; 29 in Group 1, 27 in Group 2 and 34 in Group 3. Parameters assessed were HbA1 c, FPG, blood glucose profile, Repaglinide requirements, lipid profile, change in body weight and hypoglycaemic episodes.
**Results:** HbA1 c values during the study decreased in each Group (– 0.95 ± 1.33%; p<0.001 in Group 1; -0.78 ± 1.40%; p=0.0056 in Group 2; –1.01 ± 1.12% in Group 3). No any difference between groups of adjusted means assessed by ANCOVA. Also FPG, Pre-prandial and Post-prandial glycemia decreased during the study in all three Groups without any significant difference between Groups. A slightly increase in body weight (1.3 ± 2.8 Kg; p=0.0104 vs Baseline) was recorded in Group 1 while was stable during the study either in Group 2 or 3. No any significant variation in the lipid profile for all groups. The hypoglycaemic episodes were 85, 22 and 8 respectively for Group 1, 2 and 3 even if the most of them were recorded as moderate. Hypoglycaemic episodes were experienced by 32.4, 21.2 and 11.4% of the patients respectively in Group1, 2 and 3 (p=0.1 between Groups). The Repaglinide requirements increased during the study going from 3 mg/day at baseline to 4.27 mg/day in Group 1(p<0.001), to 4.06 mg/day in Group 2 (p<0.001) and 3.71 mg/day in Group 3 (p=0.031) without any difference between three groups.
**Conclusion:** These data confirm and point out that the extemporaneous combination between Repaglinide and Metformin can be used in a variable regimen on the basis of lifestyle habits and needs of each patient respecting the efficacy, safety and tolerability profile.

## 35

**Effect of repaglinide administration on endothelial function in Type 2 diabetic patients**
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**Background and aims:** Several studies have demonstrated that endothelial dysfunction has a central role in diabetic mortality and that pro-oxidative effect of hyperglycaemia on endothelial dysfunction may actively contribute to atherogenesis. Thus, we investigate the possible effect of repaglinide and glibenclamide on endothelial function in type II diabetic patients.
**Materials and methods:** Sixteen type 2 diabetic patients volunteered for the study. The study was designed as 4-month randomized cross-over parallel group trial of repaglinide (1 mg /2 /die) versus glibenclamide (5 mg /2/die). All patients underwent the following tests: 1) anthropometrics determinations, 2) blood sampling for routine laboratory analyses and for assessment of oxidative stress indexes 3) brachial reactivity test to evaluate the endothelial function trough the study of arterial diameter and flow changes with and without intra-arterial infusion of NG- monomethyl-L-arginine, an inhibitor of NO synthase and Tetraethylammonium chloride (TEA), a $K_{Ca}$ channel blocker.
**Results:** Repaglinide and glibenclamide administration were both associated with a significant decline in fasting plasma glucose, glycosylated haemoglobin, triglycerides, FFA and with a significant increase in fasting plasma insulin and HDL-cholesterol. In addition, repaglinide administration was associated with a significant reduction in 2-hour plasma glucose levels and in degree of oxidative stress, effects not observed after glibenclamide administration. At regard brachial reactivity parameters, repaglinide, but not glibenclamide, was associated with a significant improvement in brachial reactivity parameters as shown by the increase in changes in diameter and in flow (p<0.001 and p<0.003 respectively). Moreover, intra-arterial infusion of L-NMMA and TEA slightly smoothed the beneficial effect of repaglinide.
**Conclusion:** Repaglinide administration is useful to improve brachial reactivity and to decline oxidative stress index.

## 36

**The risk of myocardial infarction and case fatality in users of antidiabetics**
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**Background and aims:** Sulfonylurea use could contribute to cardiovascular risk. Several sulfonylureas, but not new sulfonylureas (glimepiride and gliclazide), are able to block ATP-sensitive potassium channels in the heart, which prevents myoprotective effects of ischemic preconditioning. Hence, we examined if risk of myocardial infarction (MI) and the case fatality rate (CFR) differed in users of various antidiabetics.
**Materials and methods:** First, we performed a case-control study, using 6,738 cases of first-time hospitalization for MI, and 67,374 age- and gender-matched population-based controls during 1991-2002, using data from the Hospital Discharge Registry and the Civil Registration System of North Jutland County, Denmark. Next we assessed the 30-day CFR of MI patients. All prescriptions for antidiabetics prior to the hospitalization for MI were identified through a prescription database. Conditional logistic regression was used to estimate odds ratios (OR) associated with antidiabetic use (case-control study) and logistic regression to estimate relative risks of case fatality (follow-up study), adjusting for potential confounding factors.
**Results:** The risk of MI was higher in old sulfonylurea users (OR=2.07, 95% CI: 1.81-2.37) than in new sulfonylurea users (OR=1.36, 95% CI: 1.01-1.84). The overall 30-day CFR was 24.6%. The CFR was decreased among users of gliclazide (9.5%, OR=0.30, 95% CI: 0.07-1.32) and non-sulfonylurea oral

antidiabetics (22.6%, OR=0.69, 95% CI: 0.27-1.76). Users of any other antidiabetic had CFRs of 30–37%.
**Conclusion:** The use of new sulfonylureas appears to be associated with a lower risk of MI than use of old sulfonylureas. The CFR is decreased among users of gliclazide and non-sulfonylurea oral antidiabetics.

# OP 7

# Prediction and prevention of Type 2 diabetes

## 37

**Genetic prediction of the dysmetabolic syndrome: results from the Botnia study**

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**Background and aims:** The dysmetabolic syndrome (DMS) is a complex disease characterized by defects in glucose and fat metabolism resulting in clustering of metabolic abnormalities (insulin resistance, obesity, dyslipidemia, hypertension, microalbuminuria). Underlying genetic susceptibility to DMS has not hitherto been studied extensively. The aim of the present study was to evaluate whether common variants in genes involved in regulation of glucose and fat metabolism can predict development of DMS in a large prospective study (the Botnia Study).
**Subjects and methods:** In total 2293 non-diabetic individuals, of whom 1816 with no DMS at baseline (787 males/1029 females; age 44 ± 13 years; BMI 26 ± 4 kg/m$^2$; 75% with NGT and 25% with IGT) participating in the prospective study were genotyped for variants in peroxisome proliferator-activated receptor gamma (*PPARγ* Pro12Ala), Calpain-10 (*CAPN10* SNP-43, -44), muscle glycogen synthase (*GYS1* XbaI), $\beta_1$-, $\beta_2$- and $\beta_3$-adrenergic receptor ($\beta_1$-*AR* Gly389Arg; $\beta_2$-*AR* Arg16Gly; $\beta_3$-*AR* Trp64Arg) and adiponectin (*APM1* SNP276,-2019) genes. The DMS was defined according to the WHO 99 criteria. Association of the genetic variants with the onset of DMS was analysed using uni- and multivariate Cox proportional hazards model stratified on sex.
**Results:** During a median 5.5-year follow-up, 334 individuals (18%) developed DMS. In the univariate analyses, the $\beta_1$-*AR* Gly389Arg (Gly/Gly-genotype) and $\beta_2$-*AR* Arg16Gly (Arg/Arg-genotype) polymorphisms were associated with increased risk of developing DMS (HR; 1.5 [1.1-2.2] p=0.024 and 1.4 [1.1-1.7] p=0.014, respectively). The multivariate analysis of combined effects of different genotypes revealed an additive effect of $\beta_1$-*AR* Gly389Arg and $\beta_2$-*AR* Arg16Gly (2.0 [1.3-3.0] p=0.0060). Furthermore, when analyses were carried out including individuals with DMS at baseline, considering them as the fastest progressors to DMS, the *PPARγ* Pro12Ala (Ala-allele) and *APM1* SNP2019 (insertion-allele) were significant predictors of DMS (1.4 [1.1-1.6] p=0.0011 and 1.3 [1.0–1.7] p=0.025, respectively), though neither variation in the $\beta_1$-*AR* nor $\beta_2$-*AR* genes reached statistical significance. None of the variants in *CAPN10* and $\beta_3$-*AR* genes were associated with increased risk for DMS.
**Conclusion:** We demonstrate for the first time in a large prospective study that variants in the $\beta_1$-*AR* and $\beta_2$-*AR* as well as in *PPARγ* and *APM1* genes predict DMS.

## 38

**PPARγ2 Pro12Ala polymorphism and development of Type 2 diabetes in a French population, the D.E.S.I.R. study**

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**Background and aims:** PPARγ2 is a transcription factor involved in adipogenesis and insulin sensitivity. Also adiponectin may be a link between PPARγ2 and insulin resistance. The purpose of the present study was to examine whether Pro12Ala polymorphism was associated with the incidence of type 2 diabetes (T2D) or impaired fasting glycemia (IFG) in a nested case-control study of a general population followed during a three-year period. Furthermore, the effect of Pro12Ala polymorphism on adiponectin levels was estimated.
**Materials and methods:** Studied subjects participated to D.E.S.I.R (Epidemiologic Data on Insulin Resistance Syndrome) French cohort study. Of 3948 normoglycemic subjects at baseline, 229 subjects had IFG (6.1 mM ≤ fasting plasma glucose < 7.0 mM) or T2D (fasting plasma glucose ≥ 7 mM) after three years. Cases were matched for sex, age, and BMI with 229 controls with normoglycemia at three years. Genotyping used PCR DNA amplification followed by digestion with MspI restriction enzyme. Baseline plasma adiponectin levels were measured by radioimmunoassay (Linco

Research, St Charles, MO, assay sensitivity 1 ng/ml, intra- and inter-assay CV : 4.5% and 9.5%, respectively)
**Results:** PPARγ2 12Ala frequency was lower in subjects developing T2D after three years (12Ala frequency = 0.02) than in normoglycemic subjects (12Ala frequency = 0.10) or IFG subjects (12Ala frequency = 0.12) (odds ratio for TD2 vs. normoglycemic + IFG = 0.138, $p < 0.05$). In subjects developing IFG and carrying 12Ala, adiponectin was significantly higher compared to those homozygotes for Pro/Pro (26.0 ± 1.8 μg/ml vs. 24.2 ± 0.9 μg/ml, $p = 0.05$). There was no difference in adiponectin levels according to Pro12Ala polymorphism in subjects remaining normoglycemic after three years.
**Conclusion:** The PPARγ2 12Ala allele protects from type 2 diabetes but not from impaired fasting glycemia in this prospective study. Nevertheless since Ala increases adiponectin in impaired fasting glycemia patients, this protein could prevent them from developing type 2 diabetes.

## 39

**Urinary sodium, potassium excretion and the risk of Type 2 diabetes: a prospective study**
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**Background and aims:** No studies of salt intake and risk of type 2 diabetes have been reported. We aimed at finding out whether high salt intake, measured by the 24-hour urinary sodium and potassium excretion, is an independent risk factor for type 2 diabetes.
**Materials and methods:** This prospective study followed 932 Finnish men and 1003 Finnish women aged 35-64 years with complete data on 24-hour urinary sodium and potassium excretion and other study parameters. Hazard ratios for the incidence of type 2 diabetes were estimated for different levels of 24-hour urinary sodium and potassium excretion and sodium to potassium ratio.
**Results:** During a mean follow-up of 18.1 years, there were 129 incident cases of type 2 diabetes. The multivariate-adjusted (age, sex, study year, body mass index, systolic blood pressure, antihypertensive drugs treatment, smoking, coffee consumption and physical activity) hazard ratio of diabetes for the highest *vs.* combined lower quartiles of 24-hour urinary sodium excretion was 1.85 (95% CI 1.30-2.66). The hazard ratio of diabetes for the highest *vs.* combined lower quartiles of 24-hour urinary sodium to potassium ratio was 1.78 (95% CI 1.25-2.55). This trend persisted when stratified for sex, obesity and hypertension.
**Conclusion:** High sodium intake and high sodium to potassium ratio predicted the risk of type 2 diabetes, independent of other diabetic risk factors including blood pressure and body mass index. These results provide direct evidence of the harmful effects of high salt intake in the adult population.
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## 40

**The impact of the endometrial hyperglycemic environment in the offspring of diabetic mothers**
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**Background and aims:** The importance of the intrauterine environment for the development of adult disease is well recognized. The aim of this study was to investigate the impact of the hyperglycemic endometrial environment on the development of metabolic disturbances in the offspring of diabetic mothers.
**Materials and methods:** Twenty eight offspring of mothers with type 1 Diabetes (OMDM1), 40 offspring of mothers with Gestational Diabetes (OMGDM) and 85 controls (N), all aged 5–9 yrs were examined. Their height, weight, BMI and blood pressure were measured. All the children underwent an oral glucose tolerance test (1.75 g gluc/Kg). Glucose and insulin were determined at 0, 30 &120 min after glucose load. Basal insulin resistance index (HOMA) and the index of insulin secretion (IIS) were calculated. TChol, triglycerides, HDL, LDL, Lp(a), Apo (A) and Apo (B), as well as GAD 65 and IA -2A antibodies were measured. For statistical analysis ANOVA and binomial test were used.
**Results:** In the offspring of type 1 diabetic mothers the prevalence of IGT (according to the latest ADA criteria) was 25%, significantly higher ($p<0.001$) than in the other two groups (OMGDM: 7% and N: 5%). One child of this group, who also was positive for the GAD- 65 and IA -2 antibodies, presented with type 1 diabetes. The HOMA index was significantly higher in the offspring of type 1 diabetic mothers (HOMA: mean+sem : OMDM1: 2.3+0,44, OMGDM: 1.5 + 0,25, N:1.3+0,17, $p<0.05$). The index of insulin secretion (IIS) was significantly lower in both groups of offspring of diabetic mothers compared to controls (IIS: mean+sem :OMDM1:1.5+0,3, OMGDM: 1.1+0,27, N:2.2+ 0,51, $p<0.005$). The diastolic blood pressure was higher in both groups of infants of diabetic mothers (DBP:OMDM1: 65+12, OMGDM: 64+12 vs. N:55+13 mm Hg, $p<0.001$) while systolic blood pressure was higher only in OMDM1 (SBP: OMDM1: 97+12 vs. N:90+16 mm Hg). The BMI didn't differ among the groups and a similar high prevalence of overweight and obesity were observed in all three groups(overweight: OMDM1: 23.8%, OMGDM: 25.8%, N: 32.9%, obese: OMDM1: 28.6%, OMGDM: 19.4%, N: 21.2%). There were no differences in lipid parameters among groups.
**Conclusion:** The offspring of mothers with type 1 diabetes, had already at this very young age a significantly higher prevalence of IGT, and presented insulin resistance and insulinopenia, as well as high Systolic BP and Diastolic BP compared to controls. The offspring of mothers with GDM had insulinopenia and higher DBP. These early abnormalities associated with the future development of type 2 diabetes, were more prevalent in the offspring of type 1 diabetic mothers, a group in which the genetic predisposition to type 2 diabetes is not expected to be higher than in the general population . This constitutes a strong argument for the significant role of the hyperglycemic endometrial environment in the fuel-mediated transmission of diabetes in the next generations.

## 41

**Dissociation in the behaviour of body fat and insulin resistance before puberty**
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**Background and aims:** Type 2 diabetes is now presenting in adolescence and childhood. It is thought to result from a combination of insulin resistance (IR) and inadequate beta cell response. IR is also believed to drive a further series of metabolic disturbances that collectively raise the risk of cardiovascular disease (CVD). A major factor in the process is accumulation of body fat. These relationships have been largely confirmed in adults, but have not been studied closely in young children, where the disturbance may nevertheless begin.
Our aim is to explore the emerging relationships between body fat, IR and CVD risk variables in a cohort of healthy pre-pubertal UK children. We wanted to examine the behaviour of IR with age, its relation to changes in the body fat thought to cause it, and to the metabolic variables it is believed to control.
**Materials and methods:** A large cohort of healthy children was recruited at school entry (mean age 4.9, sd=0.3) from 54 primary schools across the city of Plymouth, UK, representing a wide socio-economic range. Data from 290 children, 159 boys and 131 girls, measured at baseline and annually for the following 2 years, are presented here. Measures include anthropometric proxies for body fat (waist, BMI, skinfolds), IR (measured by HOMA) and metabolic markers of CVD risk (triglycerides, blood pressure and SHBG).
**Results:** All indices of body fat rose progressively and significantly between 5y and 7y (boys $p<0.05$, girls $p<0.001$). Insulin levels and HOMA-IR, on the other hand, unexpectedly fell, significantly and substantially (boys IR=0.66, 0.51, 0.47, girls IR=0.89, 0.73, 0.60, boys and girls change from 5y to 7y $p<0.001$). Markers of metabolic risk tended to fall, though not significantly. Correlations between indices of fatness and IR strengthened rapidly with age, despite the progressive fall in IR. Correlations between IR and the metabolic variables, weak at 5y, also became stronger (table)
**Conclusion:** The data show metabolic dissociation – a progressive rise in body fat as puberty approaches, but a progressive fall in IR. Given the age-related trends in fatness and IR seen in adults, the inverse relationship between the two is counter-intuitive, but there is little longitudinal evidence in the paediatric literature to compare. The strengthening correlations between indices of body fatness and IR as children age are not incompatible with the opposite directions of their respective changes over time. Each merely indicates the strength of association at the particular time-point in question. Mechanisms are uncertain, but the deceleration of growth and fall in growth hormone prior to the onset of puberty should be considered. The immediate importance of these observations is that any intervention designed to improve metabolic profile at this age group is open to misinterpretation.

Table: Insulin Resistance (HOMA-IR) correlations with body fat indices and metabolic markers

| | 5y | | 6y | | 7y | | Change (5y vs. 7y) | |
|---|---|---|---|---|---|---|---|---|
| | Boys n=159 | Girls n=131 | Boys n=150 | Girls n=119 | Boys n=149 | Girls n=115 | Boys | Girls |
| Body Fat indices | | | | | | | | |
| Waist | 0.21** | 0.31** | 0.14 | 0.22 | 0.37** | 0.51** | 0.56 | 0.06 |
| BMI | 0.15 | 0.24** | 0.12 | 0.23* | 0.36** | 0.53** | 0.05 | 0.01 |
| Skinfolds | 0.10 | 0.12 | 0.07 | 0.16 | 0.30** | 0.55** | 0.07 | <0.001 |
| Metabolic Markers | | | | | | | | |
| Triglycerides | 0.11 | 0.17* | 0.21** | 0.11 | 0.30** | 0.34** | 0.08 | 0.16 |
| SHBG | −0.09 | −0.15 | −0.18* | −0.12 | −0.07 | 0.33** | 0.86 | 0.14 |
| Systolic Blood Pressure | −0.01 | 0.11 | 0.10 | 0.06 | 0.28** | 0.38** | 0.01 | 0.02 |
| Diastolic Blood Pressure | 0.02 | 0.02 | 0.15 | 0.1 | 0.45** | 0.24** | <0.001 | 0.08 |

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## 42

**Risk scores for Type 2 diabetes can not be applied across populations**

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**Background and aims:** Risk scores based on phenotypical characteristics to identify individuals at high risk of having diabetes are developed. They have been developed in Caucasians only. The impact of known risk factors for type 2 diabetes on the risk of having type 2 diabetes may differ between populations with different ethnic origin, and therefore it is likely that the developed risk scores can not be applied to other ethnic groups than Caucasians.
The aim of this study was evaluate the performance of two developed risk scores in the DETECT-2 Population.
**Material and methods:** Centres worldwide were invited to participate in the DETECT-2 project. The minimum requirements for participation were populations-based surveys which included information on at least 500 people without known diabetes having a 75 g oral glucose tolerance test. To date 50 centres have contributed with data including 170.000 individuals from 25 countries. In this analysis 8 cross-sectional studies were selected, which represents people with diverse ethnic and regional backgrounds. A risk score from Holland (HRS) including information on age, gender, BP treatment and BMI, and an American risk score (ARS) including age, BMI and family history of diabetes were evaluated.
**Results:** In total 33529 individuals were included in the analysis including 3501 individuals with previously undiagnosed diabetes (NDM). The table is showing the performance of the two risk scores with respect to the area under the receiver operating characteristic curve (AUC), sensitivity, specificity, positive predictive value (PPV) and the test positive fraction.

| Study | Total/ N NDM | Risk score | AUC | Sensitivity | Specificity | PPV | Test positives |
|---|---|---|---|---|---|---|---|
| Spain | 1588/164 | HRS | 0.63 | 54 | 70 | 16 | 33 |
| | | ARS | 0.70 | 70 | 60 | 15 | 43 |
| Denmark | 6006/265 | HRS | 0.60 | 33 | 85 | 9 | 16 |
| | | ARS | 0.73 | 65 | 72 | 9 | 29 |
| USA | 3120/411 | HRS | 0.64 | 64 | 59 | 17 | 44 |
| | | ARS | 0.68 | 77 | 49 | 17 | 54 |
| Australia | 9431/382 | HRS | 0.67 | 55 | 72 | 8 | 29 |
| | | ARS | 0.75 | 75 | 63 | 8 | 38 |
| South Pacific | 873/187 | HRS | 0.52 | 51 | 52 | 19 | 48 |
| | | ARS | 0.59 | 39 | 73 | 24 | 29 |
| Korea | 8655/729 | HRS | 0.55 | 34 | 79 | 12 | 22 |
| | | ARS | 0.63 | 45 | 74 | 13 | 28 |
| India | 7237/1350 | HRS | 0.55 | 20 | 87 | 23 | 14 |
| | | ARS | 0.61 | 24 | 86 | 25 | 15 |
| Africa | 1705/13 | HRS | 0.57 | 23 | 84 | 1 | 16 |
| | | ARS | 0.70 | 23 | 87 | 1 | 13 |

The AUC was higher in studies including Caucasians than other ethnic groups. The performance with respect to sensitivity, specificity, and test positive fraction was generally higher in white Caucasian populations than in other ethnic groups. Furthermore the between-test concordance differed markedly between and within populations.
**Conclusion:** This study showed that the developed risk scores can not be applied to other populations without modifications.

# OP 8
# Diabetes in childhood

## 43

**Independent effects of socio-economic status and place of residence on the incidence of Type 1 diabetes in Australian children**

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**Background and aims:** The incidence of childhood Type 1 diabetes varies by over 350-fold between countries worldwide. Regional variation in the incidence between urban and rural areas within some European countries has also been reported. There are inconsistent findings on the relationship between socioeconomic factors, which may account for some of this geographical variation, and the incidence of Type 1 diabetes.
Western Australia has a land area of over 2.5 million hectares and a population of 1.9 million that is unevenly distributed throughout the State. As Princess Margaret Hospital is the only tertiary referral centre for childhood diabetes in Western Australia, case ascertainment levels of >99% can be achieved and the characteristics of new cases accurately described.
The aim of this study was to analyse the incidence of Type 1 diabetes in 0–14 year olds in Western Australia, from 1985 to 2002, by region and socioeconomic status.
**Materials and methods:** Primary case ascertainment was from the prospective population-based Western Australia Diabetes Register and secondary case ascertainment was from the Western Australia Hospital Morbidity Data System. The case ascertainment rate was calculated using the capture-recapture method.
The postcode at diagnosis was used to categorise cases into metropolitan, rural and remote areas according to definitions used by the Western Australia Department of Health. Using the address at diagnosis, cases were categorised into 5 socioeconomic groups based on the Socioeconomic Index for Area indices published by the Australian Bureau of Statistics. Population data published by the Australian Bureau of Statistics was used as the denominator data.
Poisson regression modelling was used to analyse the incidence rates and trends, by area and socioeconomic status.
**Results:** From 1985 to 2002 there were 1144 new cases of Type 1 diabetes, of which 904 occurred in metropolitan areas, 190 in rural areas and 50 in remote areas. The case ascertainment rate was estimated to be 99.8% complete.
The mean annual age-standardised incidence was 18.3 per 100,000 person years (95% CI 17.1–19.5) in metropolitan areas, 14.4 per 100,000 (95% CI 12.4–16.5) in rural areas and 8.0 per 100,000 (95% CI 5.8–10.2) in remote areas. The incidence was significantly higher in metropolitan compared to rural areas (Incidence rate ratio (IRR) 1.27 (95% CI 1.09–1.48)) and in rural compared to remote areas (IRR 1.80 (95% CI 1.31–2.47)).
The incidence was greater with higher socioeconomic status. The incidence in the highest socioeconomic group was 72% greater than the lowest socioeconomic group (IRR 1.72 (95% CI 1.43–2.06)).
These differences in incidence by socioeconomic status and region were independent of each other.
**Conclusion:** Both higher socioeconomic status and residence in metropolitan areas are independently associated with an increased risk of Type 1 diabetes in Australian children.

## 44

**Autoantibodies rather than HLA Haplotypes predict poor residual beta-cell function during remission in children and adolescents with newly diagnosed Type 1 diabetes. Data from the Hvidøre Study Group on childhood diabetes**

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**Background and aims:** To investigate autoimmune activity (ICAs, GADAs, and IA-2As) in addition to insulin antibodies and HLA haplotypes as predictors of residual beta cell function during remission in children and adolescents.
**Material and methods:** Clinical information and blood samples were collected from 275 children and adolescents age < 16 years with newly diagnosed type 1 diabetes. Year of birth, sex, and insulin dose were recorded. HbA1 c and C-peptide were analysed centrally. A stimulated C-peptide Boost-test was carried out in each subject at 1, 6 and 12 months after diagnosis and serum for immunology and HLA typing was collected.
**Results:** ICA, GAD and IA2 mostly decreased over the 12-month period. One month after diagnosis 90% of the children were positive for at least one of the 3 autoantibodies and 86% after 12 months. Insulin antibody positivity increased from 1 to 6 months and only 1.5% were negative for this antibody at 12 months. At 12 months C-peptide level were 46% lower ($p<0.001$) and the HbA1 c significantly higher (0.63%, $p<0.05$) in addition with daily insulin dosage (0.15 U/kg/24 h, $p< 0.002$) in those with 3 autoantibodies. At 6 and 12 months ($p<0.001$) older children (11-16 yrs) tested more frequently positive for GAD antibodies. High levels of insulin antibodies at 12 months were significantly ($p<0.001$) associated with a higher dose (0.23U/kg/24 h) of exogenous insulin. Total number of pancreatic antibodies was not associated with high-risk HLA haplotypes.
**Conclusion:** Positivity for 3 pancreatic islet cell autoantibodies at 12 months was associated with poor beta-cell function and high levels of insulin antibodies were associated with increased daily insulin requirement. There seemed no association between HLA risk haplotypes and residual beta-cell function at 12 months after diagnosis.

## 45

**Influence of insulin secretion and insulin resistance on clinical course of Type 1 diabetes mellitus in children and adolescents**

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**Background and aims:** Increasing incidence of type 1 and type 2 diabetes mellitus is now observed in childhood population. More frequent prevalence of obesity and decreasing physical activity cause new interest in the role of insulin resistance in development and clinical course of type 1 diabetes mellitus in children and adolescents. Aim of this study was to estimate the influence of insulin secretion and insulin resistance on clinical course of type 1 diabetes mellitus in children and adolescents.
**Materials and methods:** 210 patients with type 1 diabetes mellitus (123 male) aged 7.3–20.3 years (mean – 13.55 ± 3.2 years) were included into study. The patients were divided in four groups according to duration of diabetes: group 1 (N=31) – 5 days, group 2 (N= 64 individuals) - 6 month, group 2 (N = 56) - 2 years and group 3 (N = 55) – 3–5 years. Insulin secretion was estimated on the base of the serum C-peptide concentration in glucagon test: C-peptide-0' fasting and C-peptide-6' in 6 minute after stimulation by glucagon (radioimmunoassay). Euglycemic-hyperinsulinemic clamp was performed to asses insulin resistance. Glucose disposal rate (index M) determined during the last 30 min of the test estimated insulin resistance. HbA1 was examined by HPLC. Body mass index (BMI) and daily dose of insulin (DDI) were calculated.
**Results:** In children and adolescent with type 1 diabetes mellitus C-peptide-0' level was 0.20 ± 0.16 pmol/l (in group 1; 2; 3; 4 - 0.26; 0.30; 0.15; 0.11 pmol/l respectively, $p<0.001$), and C-peptide-6' was 0.32 ± 0.27 pmol/l (in group 1; 2; 3; 4 - 0.44; 0.50; 0.20; 0.17 pmol/l respectively, $p<0.001$), the index M ranged from 2.4 to 17.4 mg/kg/min, mean was 7.54 ± 2.61 (in group 1; 2; 3; 4 - 7.12; 8.1; 7.36; 7.0 mg/kg/min respectively, NS). The DDI was 0.79 ± 0.30 U/kg (in group 1; 2; 3; 4 - 0.80; 0.64; 0.80; 0.96 pmol/l respectively, $p<0.001$), HbA1 c - 7.8 ± 2.21% (in group 1; 2; 3; 4 - 11.9; 6.5; 7.2; 7.5 pmol/l respectively, $p<0.001$), BMI- 0.08 ± 1.06 SD Score (in group 1; 2; 3; 4 - –0.08; 0.7; 0.27; 0.12 respectively, $p<0,001$). There was significant relationship between DDI and insulin secretion and insulin resistance (multiple regression $R=0.46$; $R2=0.21$; $p<0.001$) in all patients. The DDI correlated with insulin secretion in group 2, 3, and with insulin resistance in groups 2–4. After excluding patients with new-onset of T1DM the slight relationship between HbA1 c and insulin secretion was found (C-peptide-0' r=-0.3; p=0.001, C-peptide-6' r=-0.28; p=0.006). Higher insulin secretion and better insulin sensitiveness was observed in patients with remission of the diabetes to compared with children without remission (C-peptide-0' 0.33 v. 0.23 pmol/l, $p<0.04$; index M 9.1 v. 7.7 mg/kg/min, $p<0.05$). There was found correlation between insulin resistance and BMI (r=-0.3, $p<0.001$), but not with insulin secretion.
**Conclusion:** In type 1 diabetic children and adolescents DDI depends on their own insulin secretion and insulin resistance. The metabolic control only slightly depends on insulin secretion. Patients with remission had better insulin secretion and sensitiveness.

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## 46

**Stimulated C-peptide after 12 months of diabetes - prospective follow-up of 275 children and adolescents with Type-1 diabetes from 18 centers in Europe and Japan: results from the Hvidore Study Group**

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**Background and aims:** At diagnosis, paediatric patients with type 1 diabetes present with various degrees of metabolic derangement. Large, prospective multicenter studies are necessary to describe the relationship to the subsequent course of the disease.
**Patients and methods:** 18 paediatric centres from 15 countries in Europe and Japan collaborated for this survey. Blood samples and information were collected from 275 patients with newly diagnosed diabetes between August 1999 and December 2000. Year of birth, sex, duration of symptoms, height, weight, insulin regimen and duration of hospital admittance were recorded. Blood glucose, pH/$HCO_3$ and urinary ketones were measured locally, while $HbA_{1c}$ and C-peptide was shipped to Denmark and analysed centrally. A stimulated C-peptide Boost-test was carried out in each subject at 1, 6 and 12 months after diagnosis.
**Results:** There were 144 females and 131 males, mean age at diagnosis was 9.6 years (range 0.2 - 16.8 years). 84% of patients were white Caucasian. The mean duration of symptoms was 3.3 weeks for polydipsia and 3.2 weeks for polyuria, the average weight loss was 4 kg. At initial examination 4.7% had impaired consciousness, mean blood glucose level was 24.3 mmol/l and mean HbA1 c 11.2%. Significant ketonuria was present in 78% of patients, ketoacidosis was recorded in 20.7% and severe ketoacidosis ($HCO3^- < 10$) in 6.2% of patients. Ketoacidosis was most prevalent in young children (< 5 years: any: 28%, severe 12.5%) compared to the age groups 5–10 years (22% / 4%) and > 10 years (19% / 7%). In addition, initial BG was higher in patients < 5 years (28.0 mmol) compared to patients 5–10 years (23.0 mmol) and ≥ 10 years (24.0 mmol). 88.7% of patients were hospitalised for an average of 8.3 days, 39.1% of patients were admitted for 10 days or longer. 70% of patients with DKA received intravenous insulin, compared to 26% of patients without DKA at onset. After 1 month, 2 children (0.7%) were in complete remission (no exogenous insulin requirement), 57 received conventional therapy with 1 or 2 daily insulin injections, 22% received 3 and 15.6% were on 4+ insulin injections or pump therapy. By this time-point, HbA1 c decreased to 9.0%. Stimulated C-peptide after 1 year of diabetes could be predicted by young age, ketoacidosis (standard bicarbonate at onset) as well as C-peptide-reserve at 1 month. Neither initial blood glucose nor HbA1 c at onset were significantly related to residual β-cell function after 12 months. In contrast, metabolic control after 1 year, as assessed by HbA1 c, was only associated with HbA1 c at onset, while age, gender, initial blood glucose, initiation of insulin therapy (i.v. versus s.c.) and daily insulin dose at 1 month had no significant effect.
**Conclusion:** Today, diabetic ketoacidosis is rare at diabetes onset in Europe and Japan, but the rate is higher in younger children. In this international study, the majority of paediatric patients with type-1-diabetes was hospitalised upon diagnosis, especially in the young age-group. β-cell-function continues for a longer period in children without DKA at onset, and in older patients. Our data found no long-term benefit of intravenous insulin therapy compared to subcutaneous insulin administration during the first days of therapy.

## 47

**Metabolic control as reflectet by HbA1c in children, adolescents and young adults with Type-1 diabetes mellitus: combined longitudinal analysis including 24,147 patients from 180 centers in Germany and Austria during the last decade**

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**Background and aims:** While the central role of HbA1 c levels for the prediction of micro- and macrovascular complications in patients with type 1 diabetes is generally accepted, recommendations in current guidelines and the level of metabolic control actually achieved during routine care differ widely. Limited information is available on factors that influence metabolic control in the pediatric age-group and during the transition from pediatric to adult diabetes care.
**Material and methods:** In a large prospective multicenter database (DPV-Wiss), 307,601 individual HbA1 c measurements from 24,147 patients with type-1 diabetes (85,856 observation years) were recorded between 1994 and 2003. Data were anonymously transmitted from 180 institutions. HbA1 c-values were mathematically standardized to the DCCT normal range (4.05-6.05%). The SAS 8.2 software was used for statistical analysis using nonparametric statistics.
**Results:** Median HbA1 c for all measurements was 7.8%, with a strong effect of diabetes duration: median HbA1 c at onset was 9.1%, during the first 2 years of diabetes 7.1% with a subsequent increase to 8.0% in patients beyond the remission phase (p 2 years, 18,298 patients), a strong age-dependency was present: children 20 years: 7.4% (n=1,384; p<0.0001). HbA1 c was above recommended guidelines (adequate control: 9%) were found in 28%. For all age-groups, girls/women had higher HbA1 c values compared to boys (mean difference 0.1%, p <0.0001). Seasonal variation was remarkably small with lowest HbA1 c values in September (mean: 8.27%) and highest values in January (8.53%; p < 0.0001). No major improvement in HbA1 c was observed comparing 3 periods: 1994 - 1998, 1999 - 2001 and 2002 - 2003 (7.8% each). In a multivariate model, a significant influence on HbA1 c was detected for age (p<0001), duration of diabetes (p<0.0001), gender (p<0.02), minority status (p<0.0001), season (p<0.0001), treatment period (p<0.0001), insulin therapy (p<0.0001) and center effect (p<0.0001).
**Conclusions:** Both patient-related and treatment-related variables have a strong influence on metabolic control achieved in pediatric and young adult patients with T1DM. In contrast to a wide-spread belief, metabolic control is only marginally better in summer compared to winter. Despite considerable changes in diabetes care, no major improvement in metabolic control was observed during the last 10 years.

## 48

**Testing the accelerator hypothesis: the SEARCH for Diabetes in Youth Study**

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**Background and aims:** The Accelerator Hypothesis predicts that an earlier age at presentation of Type 1 diabetes (T1DM) is associated with fatness, potentially mediated through an intensified autoimmune response. Previous studies, however, reported discordant results. We tested this hypothesis using data from the SEARCH for Diabetes in Youth, a U.S. population-based multi-center study of childhood diabetes.
**Materials and methods:** Subjects comprised 360 youth age 0–19 years at DM diagnosis, with positive diabetes autoantibodies (DAA: either GAD65 or IA2) measured within 12 months of diagnosis, in 2003 and 2004. The relationships between age at diagnosis and fatness were examined using body mass index (BMI) at the SEARCH visit, self-reported birth weight (BWT), and weight change since birth, all expressed as standard deviation scores (SDS) based on 2000 CDC growth charts.
**Results:** BMI SDS was not significantly related to age at presentation (table), nor was it correlated with GAD65 (partial correlation coefficient, adjusted for age and DM duration, r= -0.08) and IA2 titers (r=0.04). An association between younger age at diagnosis and lower BWT SDS (p<0.001) was observed. In multiple regression analyses, age at diagnosis was also not associated with BMI SDS, controlling for DM duration, BWT and weight change since birth. Interestingly, a decrease of 1 SDS in birth weight (577 g) was associated with a 6-month earlier presentation of T1DM (p=0.02), independent of current BMI and weight change from birth.
**In conclusion,** current BMI is not associated with age at diagnosis of T1DM in this population, nor is it associated with higher DAA titers. Lower BWT SDS, as marker of reduced fetal growth and unknown in utero exposures, may have a more important impact than current BMI on the earlier age at presentation of childhood T1DM.

| Age at diagnosis quartile | N | Mean age (range) years | BMI SDS | BWT SDS | Weight change since birth SDS |
|---|---|---|---|---|---|
| 1 | 90 | 4.7 (0–6.9) | 0.78 0.63 | −0.25 −0.41 | 0.53 1.35 |
| 2 | 90 | 8.7 (7–9.9) | | | |
| 3 | 90 | 11.4 (10–12.9) | 0.64 | 0.42 | 0.83 |
| 4 | 90 | 15.1 (13–19) | 0.86 | 0.04 | 0.35 |
| p for trend | | | =0.97 | <0.001 | =0.04 |

# OP 9

# Diabetic nephropathy – mechanisms

## 49

**Enhancement in postprandial lipaemia in patients with Type 2 diabetes and microalbuminuria**

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**Background and Aims:** Microalbuminuria (MA) is an independent risk factor for atherosclerosis in patients with type 2 diabetes (T2DM). Postprandial (pp) lipaemia is also associated with accelerated atherosclerosis in both diabetic and non-diabetic subjects. The effect, however, of MA on pp lipaemia has not been studied so far. In this study we examined pp lipaemia in subjects with T2DM and MA.
**Materials and Methods:** A total of 64 patients (mean age 62.2 ± 7.2 years) with T2DM, 30 with, and 34 without MA, matched for age and sex, were examined. After 12-14 hours fast subjects received a standard mixed meal (783 Kcal: 52.5% as fat, 20% as protein, and 27.5% as carbohydrates). Plasma total triglyceride levels (Tg) were measured at baseline, and 2, 4 and 6 hours after the meal. Urinary albumin secretion was assessed by measurement (radioimmunoassay) of the 24-hour urine albumin excretion on 3 occasions over a 3-month period. MA was diagnosed when urine albumin was 30–300 mg/24 hours in 2 out of the 3 collections. Patients with macroalbuminuria or overt nephropathy were excluded. Increment of the pp plasma Tg was calculated as the difference of the pp Tg values minus the baseline values; consequently, the incremental area under the curve (AUC) was calculated using the trapezoid rule.
**Results:** Patients with MA showed almost 3.5-fold higher pp Tg in comparison to the patients without MA (mean incremental AUC ± SE): 232.4 ± 28.3 vs 69.3 ± 12.7 mg × h/dl, respectively, P<0.0001. In addition, in the patients with MA, the peak increase in plasma Tg occurred earlier (at the 2nd hour) and remained high until the end of the study.
**Conclusions:** MA is characterized by earlier and prolonged pp increase in total triglycerides in patients with T2DM. This effect of MA, which is described for first time, may explain in part the higher cardiovascular risk in these patients.

## 50

**Insulin resistance as a progression promoter in diabetic nephropathy**

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**Background and aims:** Although insulin resistance has been associated with abnormal albuminuria, there is no information on the role of insulin resistance in the progression of diabetic nephropathy in Type 2 diabetic patients. Our aim was to evaluate insulin sensitivity in Type 2 diabetic patients in relation to the rate of progression of diabetic nephropathy in a 4 year prospective observational study.
**Materials and methods:** 32 Type 2 diabetic patients with proteinuria and retinopathy were identified (mean ± SD: age 62 ± 5 years, duration of diabetes 17 ± 8 years, BMI 30 ± 4 Kg/m$^2$). Insulin sensitivity was measured at entry into the study by using euglycaemic hyperinsulinemic (200 μU/ml) clamp technique. Renal function (GFR by $Cr^{51}$-EDTA) and proteinuria were assessed yearly. HbA1 c, lipid profile and blood pressure were assessed every four months.
**Results:** On average, diabetic patients were insulin resistant (glucose disposal rate 5.08 mg/Kg/min, normal values > 10 mg/Kg/min). To evaluate the possible role of insulin resistance in the progression of nephropathy, patients were divided into two groups according to the median value of insulin sensitivity: 16 patients have a glucose disposal rate below (GROUP 1) and 16 (GROUP 2) above 5.3 mg/Kg/min. At entry into the study, no significant differences were observed between the two groups: GROUP 1 vs GROUP 2: age (63 ± 5 yrs vs 61 ± 5), duration of diabetes (17 ± 8 yrs vs 17 ± 8), GFR (76 ± 25 ml/min/1.73 m$^2$ vs 78 ± 26), blood pressure (159/84 ± 22/14 mmHg vs 153/86 ± 17/6), HbA1 c (8.6 ± 1.9% vs 9.0 ± 1.5), cholesterol (233 ± 31 mg/dl vs 229 ± 57) and triglyceride (271 ± 152 mg/dl vs 262 ± 166) plasma levels. Proteinuria was higher in GROUP 1 (2.7 ± 2.4 gr/24 hr; p<0.05) than in GROUP 2 (1.7 ± 1.3 gr/24 hr). The decline of GFR was significantly faster in GROUP 1 (-7.2 ± 4.3 ml/year, p<0.01) than in GROUP 2 (−2.9 ± 2.4 ml/year), despite similar values of blood pressure and of HbA1 c

during the 4 year follow-up period. Multiple regression analysis with insulin sensitivity, blood pressure, HbA1 c, baseline GFR, age, duration of diabetes, lipid plasma levels and proteinuria (log-transformed) as dependent variables, identifies only the degree of insulin sensitivity as a significant predictor of GFR decline ($t = 3,860$, $P = 0.001$).
**Conclusion:** A greater insulin resistance is associated with a faster decline of GFR in Type 2 diabetic patients with overt diabetic nephropathy. Whether an improvement of insulin sensitivity may slow down the rate of progression of renal disease in Type 2 diabetic patients remains to be established.

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**Insulin resistance and microvascular complications in Type 1 diabetes: the Italian cohort of the Eurodiab Prospective Complications Study**
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**Background and aims:** Among a wide range of metabolic factors that play a role in the development of diabetic microvascular diseases, insulin resistance has attracted special attention in recent studies. To further explore in Type 1 diabetes the insulin resistance-microangiopathy link, a surrogate marker of insulin sensitivity, the estimated glucose disposal rate, eGDR (Pittsburgh Epidemiology of Diabetes Complication Study), calculated using a formula derived from euglycemic-hyperinsulinemic clamp, was applied to the Italian Cohort of the EURODIAB Prospective Complications Study. This is a cross-sectional and prospective multicentre study that includes nine Diabetes and Metabolism Outpatients Clinics throught Italy.
**Materials and methods:** The cohort consists of 978 type 1 diabetics (52% M, 48% F; mean age was $32 \pm 10$ years, diabetes duration (DD) $14 \pm 9$ years; BMI $23.2 \pm 2.7$ kg/m$^2$, HbA1 c $8.1 \pm 1.8$%) re-examined after a 7.4 year follow-up. At follow-up data of 517 and 456 subjects were available for AER and retinopathy, respectively. HbA1 c was measured with an enzyme immunoassay, urinary albumin by an immunoturbidimetric method. Retinopathy was assessed by retinal photography of two retinal fields per eye. eGDR was calculated using the equation: eGDR = 24.31–12.22 (waist hip ratio) - 3.29(hypertension status, >140/90 mmHg or on drugs) -0.57 (HbA1). HbA1 was obtained by the following regression: HbA1 = 0.503 + 1.261 HbA1 c ($r^2$=0.971, $p<0.0001$).
**Results:** At entry, prevalences of microalbuminuria (mA) and overt nephropathy (ON) were 19.3 and 6.9% (normal AER, nA, 73.8%). Background (bR) and proliferative retinopathy (pR) occurred in 32.3 and 8.6% of patients, respectively (no retinopathy, nR, 59.1%). Prevalence analysis showed that at baseline eGDR was lower in ON ($5.0 \pm 2.1$ mg/kg/min) than in mA ($6.4 \pm 2.9$) and in both groups lower than in nA ($7.6 \pm 2.2$; $p<0.0001$). Both bR ($6.6 \pm 2.4$) and pR ($6.3 \pm 2.4$) had lower eGDR than nR ($7.7 \pm 2.4$ mg/kg/min; $p<0.0001$). On stepwise regression analysis eGDR (step 1), triglyceride, systolic blood pressure (BP), diabetes duration, and waist circumference were related to nephropathy, while diabetes duration (step 1), diastolic BP, HDL and sex, but not eGDR were related to retinopathy. Upon exclusion of subjects with ON or with pR who cannot progress, we analysed factors associated with progression of microangiopathy. Nephropathy progressed in 11.3% of cases, and retinopathy in 40.5%. eGDR was lower in Progressors than non-Progressors (nephropathy: $6.5 \pm 2.4$ vs. $7.9 \pm 2.2$ mg/kg/min, $p<0.0001$; retinopathy: $7.3 \pm 2.4$ vs. $8.0 \pm 2.1$ mg/kg/min, $p<0.004$). Stepwise regression analysis showed that eGDR (step 1, $p<0.0001$) and diastolic BP ($p<0.05$) at baseline were independently related to progression of nephropathy, while eGDR, triglycerides and diastolic BP were independently related to retinopathy progression.
**Conclusion:** In Italian type 1 diabetics, insulin resistance is strongly associated with prevalent and incident nephropathy and retinopathy.

## 52

**Increased renal gene transcription of protein kinase C-β in human diabetic nephropathy: relationship to long-term glycaemic control**
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**Background and aims:** The β isoform of protein kinase C (PKC) has been implicated as a central mediator in the pathogenesis of diabetic nephropathy (DNx). Although high glucose stimulates its catalytic activity, the effects of high glucose on PKC-β gene transcription are unknown. Accordingly, we sought to determine whether diabetes may lead to increased expression of the mRNA for the PKC-β gene.
**Materials and methods:** Recent advances in molecular biological techniques now permit the quantitative analysis of mRNA from archival, formalin-fixed, paraffin-embedded tissue sections. RNA was extracted from scraped 6 micron sections of biopsy tissue and PKC-β mRNA was measured using real-time PCR. PKC-β gene expression was examined in renal biopsies (n=25) with classical histological features of diabetic nephropathy and compared with that found in normal nephrectomy controls (n=6).
**Results:** Real time PCR demonstrated a substantial increase in PKC-β mRNA in all biopsies from diabetic subjects relative to controls,(DNx: 9.93 ± 1.38; Control: $1.00 \pm 0.34$, mean ± s.e.m., $p < 0.001$). In addition, a significant correlation between renal PKC-β mRNA and HbA1 c was also observed in diabetic subjects ($R = 0.63$, $p<0.05$).
**Conclusion:**This study demonstrates: (1) PKC-β is up-regulated at the level of gene transcription in diabetic nephropathy, (2) PKC-β mRNA correlates closely with HbA1 c and may explain, in part, the relationship between glycaemic control and the progression of diabetic nephropathy, (3) archival human tissue provides a valuable resource for molecular analyses.

## 53

**C-peptide stimulates Na,K-ATPase via activation of ERK1/2 MAP kinases in human renal tubular cells**
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**Background and aims:** Accumulating evidence indicates that replacement of C-peptide in type 1 diabetes ameliorates nerve and kidney dysfunction, but the mechanism involved is incompletely understood. C-peptide shows specific binding to a G protein-coupled membrane receptor resulting in $Ca^{2+}$ influx, activation of a PKC and MAP kinase signaling pathways and stimulation of Na,K-ATPase. We determined the molecular mechanism by which C-peptide stimulates Na,K-ATPase in primary human renal tubular cells (HRTC).
**Materials and methods:** Human renal tubular cells were cultured from the outer cortex of renal tissue obtained from patients undergoing elective nephrectomy. Ouabain-sensitive rubidium ($^{86}Rb^+$) uptake was determined in intact cells. Phosphorylation of Na-pump α-subunit was assessed in experiments with metabolic labeling of cells with $^{32}P_i$ and subsequent immunoprecipitation, gel electrophoresis and autoradiography. Cell surface abundance of Na,K-ATPase was determined by Western blotting after a biotinylation and streptavidin-precipitation assay or after subcellular fractionation.
**Results:** Incubation of HRTCs with 5 nM human C-peptide at 37°C for 10 min stimulated $^{86}Rb^+$ uptake by 40% ($p<0.01$). The carboxy-terminal pentapeptide was found to elicit 57% of the intact molecule's activity. In parallel with ouabain-sensitive $^{86}Rb^+$ uptake, C-peptide increased α-subunit phosphorylation and basolateral membrane (BLM) abundance of the $Na,K^+$-ATPase $\alpha_1$- and $\beta_1$-subunits. The increase in BLM abundance of the Na,K-ATPase $\alpha_1$- and $\beta_1$-subunits was accompanied by $\alpha_1$- and $\beta_1$-subunit depletion from endosomal compartments. C-peptide action on Na,K-ATPase was dependent on ERK1/2 in HRTC. C-peptide-stimulated Na,K-ATPase activation, phosphorylation and translocation of $\alpha_1$- and $\beta_1$-subunits to the BLM was abolished by the MEK1/2 inhibitor (20 μM PD98059). Furthermore, C-peptide stimulation of $^{86}Rb^+$ uptake was also abolished by HRTC preincubation with inhibitors of $G_i$ protein activation (100 ng/ml pertussis toxin) and PKC (1 μM GF109203X). Sequence analysis of Na,K-ATPase α-subunits revealed several potential ERK phosphorylation sites. C-peptide stimulated phosphorylation of human Na,K-ATPase $\alpha_1$-subunit on Thr-Pro amino acid motifs, which form specific ERK substrates.
**Conclusion:** Our results indicate that C-peptide stimulates sodium pump activity and its translocation to BLM in human kidney cells by activation of a pertussis toxin-sensitive, PKC- and MAP kinase-dependent pathway and phosphorylation of Na,K-ATPase α-subunit on Thr residues by ERK1/2. Our findings indicate that ERK1/2 dependent Na,K-ATPase α-subunit phosphorylation is a triggering signal for the $Na^+,K^+$-ATPase stimulation in HRTC. Taken together, our findings suggest that ERK1/2 is essential for C-peptide-stimulated Na,K-ATPase activation; and in a broader perspective, ERK1/2 may serve as a universal trigger of the sodium pump activation in different tissues.
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## 54

**The role of EGF receptor tyrosine kinase in experimental diabetic kidney enlargement**

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**Background and aims:** Kidney enlargement is an early and characteristic feature of diabetes that may predict subsequent renal dysfunction. Epidermal growth factor (EGF) is a pro-proliferative and anti-apoptotic cytokine that has been associated with kidney growth. However, in order to definitively establish the role of an individual growth factor, specific intervention studies are required. Using a specific inhibitor of the EGF receptor tyrosine kinase (RTK), the present study sought to examine the role of EGF in early experimental diabetic renal growth.
**Materials and methods:** Male Sprague-Dawley rats were randomized to receive streptozotocin (diabetic) or buffer (control). Animals were further randomized to receive vehicle or the specific EGF RTK inhibitor, PKI 166 (100 mg/kg by daily gavage). Animals were examined at 2 days (n=12) or 3 weeks (n=8-10) following streptozotocin. Cellular proliferation was examined by proliferating cell nuclear antigen (PCNA) and 5-bromo-2-deoxyuridine (BrdU) immunohistochemical analysis, while apoptosis was examined by the terminal dUTP nick-end labeling (TUNEL) method and active caspase 3 immunohistochemistry.
**Results:** Experimental diabetes was associated with increased kidney weight and tubular cell proliferation. These changes were significantly attenuated by treatment with PKI 166 with a 30% reduction in kidney weight ($p< 0.01$) and a diminution in tubular epithelial cell proliferation as indicated by a 60% reduction in both BrdU and PCNA staining. EGF receptor inhibition with PKI 166 was also associated with a 40% increase ($p< 0.01$) in tubular epithelial cell apoptosis.
**Conclusion:** These findings indicate that the EGF system is important in early diabetic kidney growth by effects on both cell proliferation and apoptosis. Long term studies will be needed to assiss the effects of EGFr inhibition on the development and progression of diabetic nephropathy.
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# OP 10
# Nutrition: novel concepts

## 55

**Altered body composition and metabolism in the male offspring of high fat-fed rats.**

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**Background and aim:** Exposure to a suboptimal intrauterine environment may play a pivotal role in predisposing the developing fetus to metabolic diseases in later life. Many nutritional exposures throughout pregnancy and lactation can influence the early life programming of the offspring's metabolism. In today's Western society, maternal excess fat consumption may influence this early life metabolic programming. The aim of this study was to investigate the hypothesis that a maternal high ω-6 polyunsaturated fat diet can modify the offspring's body composition and metabolism.
**Materials and methods:** 16 female Wistar rats were fed either standard laboratory chow (CON) or a high ω-6 polyunsaturated fat diet (FAT) for 4 weeks prior to breeding. These diets were continued throughout gestation. During lactation all mothers were fed laboratory chow. At 21 days of age offspring were weaned and maintained on laboratory chow for the study duration. At 3 months of age male offspring (16 CON, 15 FAT) underwent body composition measurements using dual x-ray absorptiometry. 3 days later oral glucose tolerance tests (OGTTs) (3g glucose/kg body weight) were performed. 3 days post-OGTT the quadriceps muscle and liver were removed and analysed for triglyceride content and the expression levels of key insulin signalling proteins, namely the insulin receptor β subunit, insulin receptor substrate-1 (IRS-1), the p85 subunit of PI3-kinase and PKC-ζ. GLUT-4 expression was also measured in the quadriceps muscle. Results are expressed as mean ± SEM. Insulin signalling protein expression in the tissues of the FAT offspring is expressed as a percentage of the normalised CON protein expression.
**Results:** At 3 months of age the FAT offspring had increased body (15.9 ± 0.6% c.f. 13.6 ± 0.6%, $p<0.01$) and abdominal fat (13.4 ± 0.9% c.f. 9.9 ± 0.7%, $p<0.01$). All offspring displayed normal glucose tolerance, though the FAT offspring were more hyperinsulinaemic 15 minutes after the oral glucose challenge (262.7 ± 30.8pM c.f. 151.8 ± 17.2pM, $p<0.005$). FAT offspring had elevated triglyceride content in liver (5.9 ± 0.5 μmol/g liver c.f. 4.3 + 0.5 μmol/g liver, $p<0.05$) but not in quadriceps muscle. Expression of hepatic insulin receptor β subunit and hepatic IRS-1 were reduced in the FAT offspring (53.3 ± 7.0% c.f. 100.0 ± 3.6%, $p<0.001$; 82.9 ± 4.7% c.f. 100.0 ± 4.5%, $p<0.05$). Hepatic expression of PKC-ζ was elevated in the FAT offspring (118.5 ± 4.1% c.f. 100.0 ± 2.8%, $p<0.001$). Expression of the insulin receptor β subunit and the p85 subunit of PI3-kinase were increased in the quadriceps muscle of the FAT offspring (133.3 ± 5.3% c.f. 100.0 ± 7.7%, $p<0.005$; 110.0 ± 3.1% c.f. 100.0 ± 3.4, $p<0.05$). There was also a tendency for a higher expression of the GLUT-4 muscle protein in the FAT offspring (121.7 ± 8.4% c.f. 100.0 ± 8.0%, $p=0.07$).
**Conclusion:** A maternal diet high in ω-6 polyunsaturated fat evokes detrimental early life programming. There are subsequent alterations within the body composition and important metabolic processes that are evident in the young adult offspring. Maternal high fat feeding may predispose the offspring to an increased risk of developing metabolic diseases in adult life.

## 56

**Exposure to a low protein diet in early life reduces the incidence of diabetes in NOD mice.**

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**Background and aims:** Non-obese diabetic (NOD) mice spontaneously develop diabetes following the autoimmune destruction of β-cells in the islets. However, pancreatic islet lymphocytic infiltration (insulitis) does not necessarily proceed to complete destruction of β-cells and diabetes. Progression from insulitis to diabetes in the NOD mouse is typically associated with T helper 1 (Th1) pancreatic inflammation, whereas T helper 2 (Th2) inflammation can seemingly be controlled indefinitely. The NOD mouse is an excellent model to study autoimmune diabetes associated with insulitis,

as it shares several immunopathogenic features with the disease in humans. Human and rodent studies have shown that early exposure to different sources of proteins is an environmental risk factor for the development of diabetes.
**Hypothesis:** Our aim was to examine the impact of a protein restricted diet during early life in the NOD mouse on pancreatic development, and the timing of onset of insulitis and diabetes. We also assessed the mechanistic pathway of the development of insulitis by studying the presence of Th1 cytokine (IFN-γ) or Th2 cytokines (IL-4, IL-10) and TNFα.
**Materials and methods:** NOD breeding pairs were obtained from the Robarts Research Institute (University of Western Ontario). Mice were mated and, after confirming pregnancy, were divided in two groups. The first group was fed a 20% protein diet (C, n = 7 litters), and the second one received an isocaloric 8% protein diet (LPD, n = 8 litters); after weaning (21 days-old) all pups were fed with the 20% protein diet. The pregnant mice and the pups were weighed regularly. At 8 weeks of age, normal onset of insulitis for this colony, some pups were sacrificed. The remaining pups were monitored for the onset of diabetes by checking glycosuria, and sacrificed after confirming diabetes by hyperglycemia, or at 50 weeks of age. Blood samples were collected for cytokines measurements by ELISA. Pancreata were dissected and analyzed for the presence of insulitis. Parameters as mean islet area, glucagon and insulin area were determined by immunohistochemistry followed by image analysis.
**Results:** The weight gain by females during gestation for both treatments was similar. LPD did not affect growth curve of female pups, but the weight of male pups was significantly reduced. At 8 weeks, the glycemia after 18 h fasting was 3.4 ± 0.17 mM for both diets and sexes. Preliminary data suggests that at 8 weeks, the LPD reduces the insulitis by 50% when compared to C. Mean islet area and β-cell area were also reduced in LPD group. Serum INF-γ levels were reduced by the low protein diet both at 8 weeks of age and at the onset of diabetes, while no significant changes were seen in serum IL-4 levels. A delay in the onset of diabetes was observed in both females and males from LPD group, with a reduction in the incidence of the disease; at 23 weeks, 50% of C females developed diabetes, while only 26% of the LPD females were diabetic at the same age.
**Conclusion:** The development of the endocrine pancreas was affected by the restricted diet. The reduction in the incidence of insulitis and the absence of INF-γ observed at 8 weeks of age in the low protein diet group, were reflected later, in the adulthood, by a decrease in the development of the disease. We conclude that the diet is a critical factor in diabetes development, being susceptible to nutritional changes during fetal and neonatal life.
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**Nutritional support with oral amino acid mixture improves metabolic control and insulin sensitivity in poorly controlled elderly Type 2 diabetic subjects**
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**Background and aims:** Alterations of muscle functions, strength and mass in aging can lead to decreased physical activity and glucose uptake with onset of disorders of glucose tolerance, reduced insulin sensitivity, hyperinsulinemia and diabetes. Within this context, the aim of the present study was to induce muscle anabolism and trophic conditions by increasing nutritional support with oral administered amino acid mixture (OAAM) in elderly subjects with poorly controlled Type 2 diabetes.
**Materials and methods:** A randomized open label crossover study was conducted in 40 normal-weight old subjects (age-range: 65-85 yr.) with poorly controlled (HbA1 c > 7%) Type 2 diabetes (20 with OAAM and 20 with placebo). OAAM (449 KJ calories per day; Mixture: l-leucine 2.5g, l-lysine 1.3g, l-isoleucine 1.25g, l-valine 1.25g, l-threonine 0.7g, l-cysteine 0.3g, l-histidine 0.3g, l-phenylalanine 0.2g, l-methionine 0.1g, l-tyrosine 0.06g, l-tryptophan 0.04g) and placebo were ingested as snacks at 10.00 AM and 06.00 PM, maintaining a total daily amount of calories of 1600 ± 370 Kcal (55% carbohydrates, 30% lipids, 15% proteins). The study consisted of 4 phases: 1) run-in and baseline examination (2 weeks prior to OAAM or placebo administration); 2) randomization and 16 weeks maintenance period either with OAAM or placebo; 3) 2 weeks wash-out period prior cross-over; 4) 16 weeks treatment period either on OAAM or placebo.
**Results:** OAMM treatment significatly reduced HbA1 c levels (baseline: 7.9 ± 2%, after 16 weeks: 6.5 ± 1.1%, $p<0.001$), fasting blood glucose (baseline: 7.9 ± 1.5 mmol/L, after 16 weeks: 6.5 ± 1 mmol/L, $p<0.001$), 2 h postprandial blood glucose (baseline: 9.4 ± 3.3 mmol/L, after 16 weeks: 7.2 ± 1.6 mmol/L, $p<0.001$); whereas all parameters remained unchanged in placebo group. Mean 24-h and bed-time blood glucose were also reduced during OAAM administration. Fasting serum insulin levels were slightly increased at baseline (113 ± 29 pmol/L) and significantly reduced after 4, 8 and 16 weeks of OAAM administration (98 ± 19 pmol/L, 97 ± 16 pmol/L and 94 ± 15 pmol/L respectively, $p<0.001$). HOMA-$_{IR}$ was significantly reduced during OAAM (from 5.7 to 4.1, $p<0.01$ after 8 weeks and 3.3, $p<0.001$ after 16 weeks) and also maintained at decreased levels after cross-over to placebo (3.6, $p<0.001$ after 34 weeks). Significant increase of HDL-cholesterol ($p<0.01$ after 8 weeks), of cellular IGF-1 levels ($p<0.001$ after 12 weeks) and of muscle mass (measured by DEXA) were found in OAMM group; whereas serum creatinine and urinary albumin excretion rate were unchanged during the study.
**Conclusion:** Our data suggest that nutritional supplementation with OAAM can increase amino acid availabilty for muscle biochemical functions, strenght and mass in elderly Type 2 diabetes. This mechanism could potentially improve metabolic conditions in these patients by means of increasing insulin sensitivity and of glucose uptake and utilization by muscle itself. Therefore, frailty and sarcopenia could be considered as pathogenetic risk factors for diabetes mellitus in old people.
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**The effect of mediterranean diet on risk factors for cardiovascular disease in healthy men**
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**Background and aims:** Adherence to mediterranean type of diet has been associated with reduction of the incidence of cardiovascular (C.V) disease.The aim of this study was to evaluate the effect of consumption of Greek type mediterranean diet (rich in fiber, mono-and polyunsaturated fatty acids and complex carbohydrates) on anthropometric and laboratory parameters considered risk factors for C.V disease.
**Materials and methods:** 22 men aged 48,3 ± 14,9 (M±SD) years were evaluated before and following a 28 day consumption of Greek mediterranean diet.The food,rich in olive oil,fish,vegetable and fruit,was prepared and provided daily by a commercial catering firm and was isocaloric to the current so that the study subjects would not reduce their weight during the test period. The anthropometric and biochemical parameters were assessed before and after the 28 day period on the diet. The Wilcoxon matched paired test was applied for the statistical analysis. All values are expressed as means±SEM.
**Results:** BMI did not change following the diet period however waist perimeter was significantly reduced ($p<0,05$). It was found that systolic blood pressure dropped from 124,3 ± 2,4 to 121,2 ± 2,4 mmHg ($p=0,02$). There was a significant reduction in aspartate aminotransferase (20,8 ± 0,9 versus 22,3 ± 5,2 U/L, $p<0,05$), γ-glutamyltransferace (17,1 ± 2,0 versus 17,7 ± 1,8 U/L, $p<0,05$), triglycerides (117,4 ± 10,2 versus 142,1 ± 14,5 mg/dl, $p<0,05$) and apolipoprotein B (99,1 ± 5,0 versus 114,0 ± 4,1 mg/dl, $p=0,01$).Finally insulin resistance,as assessed by HOMA, was also significantly reduced (2,2 ± 0,32 versus 5,1 ± 1,8, $p=0,01$).
**Conclusions:** It is concluded that in normal men even a short term adherence to Greek type of mediterranean diet may reduce waist perimeter, systolic blood pressure, liver enzymes, triglycerides and apolipoprotein B levels as well as lower insulin resistance, factors considered risk factors for the development of cardiovascular disease and the metabolic syndrome.
*Supported by: General Secretariat of Research*

## 59

**The effects of a 'Mediterranean' diet versus a 'Northern European' diet on glucose-treated vascular endothelial cells**
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**Background and Aim:** In diabetes, in addition to disturbances in glucose homeostasis, there is impairment of fatty acid metabolism which results in increased free fatty acids in the bloodstream. The oleic acid-rich Mediterranean diet in contrast to the linoleic acid-rich Northern European diet may protect against atherosclerosis by improving endothelial function. Fatty acids may influence endothelium function through the regulation of the expression of adhesion molecules and chemokines via the activation of the transcription factors, PPAR and NFκB. The aim of this study was to

evaluate the effects of oleic acid versus linoleic acid on the expression of adhesion molecules and activity of transcription factors in glucose-treated endothelial cells.
**Materials and Methods:** Vascular endothelial cells were isolated from porcine aorta and cultured in normal (5 mmol/l) or high (25 mmol/l) D-glucose for six days. The fatty acids, oleic and linoleic, were added to cell culture medium (final conc 0.2 mmol/l) using fatty acid free BSA as a carrier (6:1) for the final three days of culture. Total RNA was extracted using RNeasy Mini columns (Qiagen) and nuclear extracts prepared using a nuclear extraction kit (Active Motif). Total RNA was reverse transcribed and the resulting cDNA used in real time PCR on an ABI Prism 7000 Sequence Detection System. Values were normalised to the housekeeping gene, GAPDH and the comparative $C_T$ method used to quantify gene expression relative to the control (normal glucose + BSA). The activity of the transcription factors, PPARγ, and p65 (NFκB) were measured using TransAM ELISA kits (Active Motif) and all values expressed as a percentage of the control (normal + BSA).
**Results:** High glucose alone increased PPARγ (143 ± 6.4% v 100%, $p<0.05$) and p65 (194 ± 49%) binding activity which was accompanied by an increase in mRNA expression of the adhesion molecules E-selectin (1.43 ± 0.08 v 1, $p<0.05$), VCAM-1 (1.35 ± 0.33 v 1, $p<0.05$) and ICAM-1 (1.38 ± 0.5 v 1, $p<0.05$) compared to controls. The effects of oleic acid on endothelial cells was influenced by the glucose condition, that is, oleic acid decreased VCAM-1 (0.63 ± 0.07, $p<0.05$) and ICAM-1 (0.51 ± 0.18 v 1, $p<0.05$) mRNA expression in normal glucose but had no lowering effect in high glucose. Linoleic acid increased E-selectin gene expression (1.97 ± 0.33 v 1) in high glucose conditions and decreased VCAM-1 expression (0.50 ± 0.08 v 1, $p<0.05$) in normal glucose compared to the normal controls. The addition of oleic acid increased p65 binding activity (139 ± 7%, $p<0.05$) but decreased PPARγ binding activity (68 ± 9%, $p<0.05$) in normal glucose compared to controls.
**Conclusion:** High glucose modulates the effects of the fatty acids, oleic and linoleic, on transcription factor activity and adhesion molecule gene expression in aortic endothelial cells. Furthermore, oleic acid and linoleic acids have differential effects on markers of endothelial dysfunction.

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**Effects of dietary saturated, monounsaturated and n-3 fatty acids on blood pressure and haemostatic factors in healthy subjects**

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**Background:** The quantity and quality of fats consumed in the diet influence the risk of cardiovascular disease (CVD). While the impact on plasma lipids and lipoproteins is well described, less information exists on the role of fats on blood pressure and haemostatic factors.
**Aim:** To evaluate the effects of different types of dietary fat (monounsaturated vs. saturated fatty acids, and n-3 or placebo supplementation) on blood pressure and haemostatic factors in healthy subjects.
**Materials and methods:** 162 healthy subjects were randomly assigned for 3 months to follow two isoenergetic diets, one rich in monounsaturated fatty acids (MUFA diet) and the other in saturated fatty acids (SAFA diet). Each group was further randomised to receive supplementation with fish oil (3.6 g/day n-3 fatty acids) or placebo.
**Results:** Both the systolic (SBP) and the diastolic blood pressure (DBP) were lowered by the MUFA diet ((-2.2%, p=0.009) and (-3.8%, p=0.0001), respectively), but did not change on the SAFA diet. The MUFA diet caused significantly lower DBP than the SAFA diet (p=0.0475). The addition of n-3 fatty acids influenced neither SBP nor DBP. Interestingly, the favourable effects of substituting MUFA for SAFA on DBP disappeared at a total fat intake above median (>37 E%). The SAFA diet tended to increase Factor VIIc (p=0.0522), being significant (p=0.0389) at a total fat intake above median (> 37 E%). There was no difference in the effect of SAFA and MUFA on fibrinogen, von Willebrand factor, PAI-1, alfa-2 macroglobulin or prothrombin fragment F1+2 during the study. The addition of n-3 fatty acids decreased alfa-2 macroglobulin (p=0.0002) and von Willebrand factor (p=0.0198).
**Conclusion:** Changing the proportions of dietary fatty acids in favour of MUFA vs. SAFA lowered DBP and tended to decrease the Factor VIIc level. The favourable effects of substituting MUFA for SAFA disappeared at a total fat intake above median (>37 E%). The addition of n-3 fatty acids influenced neither SBP nor DBP but decreased alfa-2 macroglobulin and von Willebrand factor levels.

# OP 11

# Signal transduction in beta cell function and dysfunction

## 61

**Role of PAS kinase as a glucose sensor in pancreatic MIN6 β cells**

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**Background and aims:** The mechanisms through which glucose regulates the expression of the preproinsulin (PPI) and other genes are still not fully defined. Here, we explore the role of the recently-identified mammalian homologue of *S. cereviseae* PAS (per-arnt-sim) kinase (PASK), a member of the AMP-activated protein kinase family of nutrient-sensitive protein kinases.
**Materials and Methods:** PASK expression was assessed using semi-quantitative RT-PCR and Western (immuno-) blot analysis. PASK activity was assessed by 'SAMS' peptide kinase assay. The activities of wild-type and point-mutated PPI and PDX-1 promoters were assessed by single cell microinjection of luciferase reporter constructs and photon counting and mRNA levels by quantitative RT-PCR (TaqMan™, SYBR green). Small interfering RNAs against PASK were designed using strategies described previously and introduced into cells using Oligofectamine™. Insulin secretion was assessed by radioimmunoassay and co-transfected human growth hormone release by ELISA.
**Results:** Elevated glucose concentrations stimulated PASK activity and expression in both primary rat islets and in MIN6 cells. Thus, incubation of MIN6 cells at 30 *vs* 3.0 mM increased PASK activity by 45.0 ± 1.47% after 60 min., likely due to increased phosphorylation of threonine-1161 in the regulatory "T-loop", an event followed by the accumulation of both PASK mRNA (by 40.8 ± 0.7%) and protein (by 51.5 ± 0.672%) after 6 h. Implicating a role for PASK in the up-regulation of the insulin gene by glucose, microinjection into single cells of the purified wild-type PASK protein into primary beta cells in intact islets and MIN6 beta cells by cytosolic and intranuclear micro-injection led to significantly increased PPI promoter activity at basal 3 mM glucose (to a 2.05 ± 0.03 and 4.27 ± 0.03-fold increase respectively in primary rat β- and MIN6 cells; corresponding values for the effects of 30 mM glucose were 2.00 ± 0.003 and 9.09 ± 0.03 fold), respectively. Conversely, over-expression of a kinase-dead inactive ($K^{1028}R$) PASK kinase mutant, likely to act as a dominant-negative towards endogenous PASK, completely reversed the activation by 30 mM glucose of both PPI and PDX-1 transcription in MIN6 cells. Moreover, silencing of PASK mRNA suppressed the induction by glucose of PPI, PDX-1 and MafA mRNAs, but was without effect on the levels of mRNAs encoding HNF3β (Foxa2), HNF1α, USF, β2 / neuroD1, glucokinase or UCP2.
**Conclusions:** Changes in PASK activity regulate PDX-1 and preproinsulin gene expression and may thus play a role in specifying and maintaining the β-cell phenotype.
*Supported by : the Wellcome Trust*

## 62

**IRS-2 proteasomal degradation mediated by a mTOR-induced negative feedback downregulates PKB-mediated signalling pathway in β-cells**

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**Background and aims:** IRS-2 mediated signaling pathways play a key role in regulation of β-cell mass. We have previously shown that adenoviral-mediated overexpression of IRS-2 is increasing both β-cell proliferation and β-cell survival. In addition, specifically blocking IRS-2 expression with IRS-2 antisense induces apoptosis in β-cells. Therefore, regulation of IRS-2 protein expression is critical to maintain normal β-cell growth. In peripheral tissues, IRS-2 proteasomal degradation can lead to the downregulation of insulin signaling pathways and contributes to insulin resistance. In this study we present experimental evidence of a similar negative feedback pathway whereby IRS-2 protein is degraded, leading to a decrease in β-cell survival.
**Materials and methods:** We performed our studies in INS-1 cells and assess serine/threonine (Ser/Thr) phosphorylation of IRS-2, IRS-2 protein levels, PKB phosphorylation, and cleaved/activated caspase-9 by immunoblot analysis. Cleaved caspase-9 was also evaluated by immunofluorescence analysis. Two mutated forms of mTOR, one constitutively active form (mTORΔ) and one negative 'kinase-dead' form (mTOR-KD), were expressed in the cells by adenoviral infection.
**Results:** We find that IRS-2 protein expression is downregulated by chronic activation of mTOR signaling pathway in INS-1 cells, via proteasomal degradation. An 8 h-exposure to 15 mM of glucose or 5 nM IGF-1, induced phosphorylation of IRS-2 causing an upward mobility shift in the protein migration of IRS-2 in polyacrylamide gel, which was reduced after alkaline phosphatase treatment of the lysates, indicating a Ser/Thr phosphorylation of IRS-2. Inhibition of the 26S proteasome by lactacystin (10 μM) further increased the gel shift, but this shift was not completely inhibited after alkaline phosphatase treatment. Thus, it is likely that IRS-2 is not only Ser/Thr phosphorylated but also ubiquitinated before being degraded, contributing to a slower protein migration in the gel. Glucose/IGF-1-induced Ser/Thr phosphorylation of IRS-2 was further increased in adenoviral-infected cells expressing mTORΔ, whereas it was prevented by rapamycin (50 nM) or by adenoviral-mediated expression of mTOR-KD. An 8 h-exposure of adenoviral-infected cells expressing mTORΔ to IGF-1, decreased IRS-2 protein levels. This decrease was more significant after 24 h-exposure to IGF-1 and was prevented by mTOR inhibition (either by adenoviral-mediated expression of mTOR-KD or rapamycin) as well as inhibition of the 26S proteasome by lactacystin. Reduced IRS-2 protein expression resulted in a specific decrease of IRS-2 mediated PKB phosphorylation and an increased apoptosis.
**Conclusion:** Our results show that chronic activation of mTOR by glucose or IGF-1 increases Ser/Thr phosphorylation and ubiquitin-mediated proteasomal degradation of IRS-2. In addition, degradation of IRS-2 protein mediated by mTOR is associated with downregulation of PKB-mediated pathway, which plays a major role in β-cell survival. As such, mTOR-mediated protein degradation of IRS-2 constitutes a negative feedback mechanism for the regulation of β-cell growth, that, in turn might contribute to adversely affecting β-cell mass as a consequence of chronic hyperglycemia associated with the pathogenesis of type-2 diabetes.

## 63

**Functional heterogeneity of mitochondria in an individual beta cell**

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**Background and aims:** Beta cell mitochondria play a key role in probing and processing fuel availability to yield a series of metabolic signals for insulin secretion. Activity of mitochondrial oxidative phosphorylation, has been shown to drive insulin secretion, demonstrated by a close correlation of pulsatile insulin secretion to oscillations in oxygen consumption, beta oxidation, mitochondrial membrane potential and ATP production. To examine the metabolic response of mitochondria at the resolution of the individual organelle, we developed a methodology to allow confocal microscopy based ratiometric imaging of mitochondrial membrane potential.
**Materials and methods:** Image analysis algorithms were developed to quantify the distribution of the diverse population of mitochondria under different conditions. Mouse pancreatic beta cells, INS-1 cells and HIT cells were examined in the presence of basal and stimulatory glucose concentrations.
**Results:** In all three experimental models mitochondria were found to be divided into polarized and depolarized subpopulations. Mitochondrial hyperpolarization in response to glucose did not eliminate this heterogeneity. Membrane potential can be maintained by the activity of the respiratory chain, or by the reverse activity of the F0F1 ATPsynthase, both pumping protons out from the matrix. To determine the metabolic source maintaining the membrane potential of the different subgroups of organelles we used oligomycin, which blocks the synthesis or hydrolysis of ATP by F0F1 ATPsynthase. In the presence of oligomycin there was an overall increase in the average cellular mitochondrial membrane potential, however, even under these conditions a subpopulation of mitochondria were found to be depolarized.
**Conclusion:** The presence of this depolarized subpopulation indicates that the electrochemical gradient in these mitochondria was generated by the reverse action of F0F1 ATPsynthase. We conclude that a subpopulation of polarized mitochondria in beta cells may act as ATP consumers rather than producers. The mechanism behind the observed heterogeneity is currently being sought using a combined genetic and pharmacological approach.

## 64

**Leucine increases glucose sensitivity of rat islets via up-regulation of ATP synthase**

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**Background and aims:** As a fuel, leucine has long been known to stimulate insulin secretion of pancreatic islets and β-cell lines, stimulate β cell proliferation and enhance protein translation via the mTOR signal pathway. The aim of this study was to investigate whether leucine affects β-cell glucose sensitivity.
**Materials and methods:** Isolated rat and human islets were cultured with or without 10 mM leucine for 1 day or 1 week, respectively. Then insulin secretion in perifused and incubated islets, ATP content were assayed. Real time RT-PCR was used to assay some key metabolic genes involved in insulin secretion in islets.
**Results:** The results showed that culture with leucine for 1 day failed to affect glucose-induced insulin secretion in rat islets. In contrast, culture with leucine for 1 week resulted in decreased threshold for glucose-induced insulin secretion (shift from 6 mM to 2 mM, $p < 0.05$) and increased maximal insulin secretion at 30 mM glucose (300% of control) in rat islet. In the absence of glucose ATP content was not different ($p > 0.05$) with or without leucine culture for 1 week. In the presence of 20 mM glucose ATP content was higher in islets cultured for 1 week with leucine than those without leucine. Real time RT-PCR analysis showed that in leucine-treated rat islets, ATP synthase mRNA levels were increased after 1 week compared to control. Culture with D-leucine for 1 week failed to affect glucose-induced insulin secretion and ATP synthase mRNA level in rat islets. Treatment with leucine for 1 week increased glucose-induced insulin secretion of type II diabetic human islets. In the absence of glucose both treated and non-treated human islets had the similar insulin secretion ($12.1 \pm 2.4$ versus $11.1 \pm 3.4$ μU/islet.hour, $p > 0.05$). In the presence of 5 mM ($20.0 \pm 3.4$ versus $11.6 \pm 2.9$ μU/islet.hour, $p < 0.05$) and 20 mM ($31.4 \pm 8.8$ versus $14.3 \pm 3.7$ μU/islet.hour, $p < 0.05$) glucose treated-islets secreted more insulin than non-treated islets. Depletion of ATP synthase mRNA by siRNA cassette decreased glucose-induced insulin secretion in INS-1 cells by 50%.
**Conclusion:** Our findings revealed that the fuel-sensing role of mitochondrial ATP synthase in the control of ATP production from glucose and the control of glucose-induced insulin secretion.
*Supported by: NIH*

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**Aspartate-glutamate carrier Aralar1 plays a regulatory role in metabolism secretion coupling in INS-1E cells and rat islets**

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**Background and aims:** Aralar1 and citrin are the two known aspartate-glutamate carrier (AGC) isoforms of the malate-aspartate shuttle. Overexpression of aralar1 in the rat insulinoma INS-1E increases mitochondrial membrane potential, glucose oxidation, and insulin secretion. In the present study, we further investigated the effects of aralar1 overexpression on the correlation between metabolic parameters and insulin secretion in INS-1E cells, as well as secretory responses in isolated rat islets.
**Materials and methods:** The recombinant AdCA-Aralar1 adenovirus was used to transduce INS-1E cells and isolated rat islets. NAD(P)H autofluorescence was monitored in vivo in transduced cells. ATP concentrations were determined in cells using luciferase-mediated photon emission. Insulin secretion was measured over a 30 min glucose stimulation in INS-1E cells and rat islets, 24 hours after viral transduction, and analysed by RIA.
**Results:** The expression of aralar1 and citrin was studied by Western blot in protein extracts of adult rat tissues and INS-1E cells. Aralar1 was highly expressed in the brain and at lower levels in kidney, lung, liver, islets and INS-1E cells. Isolated rat islets did not exhibit detectable levels of citrin. Therefore, between the two known forms of aspartate/glutamate carriers, aralar1 is the one expressed in beta cells. However, the levels are lower compared to the brain, another neuroendocrine tissue. This suggested the potential of increasing aralar1 expression in beta cells. Stimulation of control INS-1E cells with 15 mM glucose increased NAD(P)H levels (+53%, $p < 0.05$). In cells overexpressing aralar1, glucose-induced elevations of NAD(P)H were augmented by +37% ($p < 0.05$) versus controls. Thus, overexpression of aralar1 potentiated NAD(P)H elevations evoked by glucose. Although dynamic cytosolic ATP changes where not affected by aralar1 overexpression, total cellular ATP levels were modified. Glucose-induced ATP increases were larger ($p < 0.05$ vs. control cells) in aralar1 overexpressing cells (+36%, $p < 0.01$) than in control cells (+18%, $p < 0.05$). These metabolic changes induced by aralar1 overexpression correlated with enhanced insulin secretion in response to 15 mM glucose (+45%, $p < 0.001$). Insulin secretion in rat islets stimulated with 16.7 mM augmented 5.5-fold ($p < 0.05$). In rat islets overexpressing aralar1, the secretory response was further enhanced by 65% ($p < 0.05$) versus corresponding control. Moreover, aralar1 overexpression rendered islets resistant to low doses of aminooxyacetate (1 mM), which inhibited significantly insulin secretion in control islets (-38%, $p < 0.05$).
**Conclusion:** Aralar1 is the AGC isoform expressed in beta cells, although at lower levels than in the brain. Aralar1 overexpression in INS-1E cells improved glucose responses in terms of NAD(P)H generation and total ATP levels, accompanied by increased insulin release. Isolated rat islets exhibited elevated secretory responses to glucose after transduction with AdCA-Aralar1 adenovirus and were less sensitive to aminooxyacetate. These results suggest that aspartate-glutamate carrier capacity is limiting coupling of glucose metabolism to insulin secretion.
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**Functional characterization of pancreatic islets isolated from Type 2 diabetic donors**

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**Background and aims:** Type 2 diabetes mellitus (T2DM) is characterized by both disordered insulin action and abnormalities of insulin secretion.
**Materials and methods:** To directly examine the function of islets from T2DM patients, we prepared isolated islets from the pancreata of 13 T2DM cadaveric organ donors (age: $67 \pm 8$ yrs; M/F: 6/7; BMI: $29.4 \pm 5.0$ kg/m$^2$; known duration of diabetes: $5.7 \pm 5.7$ yrs) and compared them with islets from 13 non-diabetic donors (age: $62 \pm 11$ yrs; M/F: 9/4; BMI: $27.3 \pm 2.1$ kg/m$^2$).
**Results:** Insulin secretion (expressed as stimulation index, i.e. the ratio of stimulated release over basal release) from T2DM islets was significantly lower than from control islets in response to glucose (SI: $1.2 \pm 0.4$ vs $2.2 \pm 0.8$, $p < 0.01$). Insulin secretion from T2DM islets was better maintained upon stimulation with arginine (SI: $1.7 \pm 0.5$, $p < 0.05$ vs glucose-stimulated SI) or glibenclamide (SI: $1.8 \pm 0.5$, $p < 0.05$ vs glucose-stimulated SI). Insulin mRNA expression was significantly lower in T2DM than in control islets ($-93.8 \pm 4.7\%$, $p < 0.01$). In addition, in T2DM islets the expression of Glut-1, Glut-2 and glucokinase was also reduced ($-35 \pm 4.9\%$, $-48.2 \pm 3.5\%$ and $38.5 \pm 6.4\%$, respectively, all $p < 0.05$), whereas that of phosphofructokinase, aldolase and pyruvate kinase was unchanged, and that of glyceraldehyde-3-phosphate dehydrogenase was increased ($+90 \pm 6.8\%$, $p < 0.05$). Nitrotyrosine and 8-hydroxy-2'-deoxyguanosine concentrations, which are considered markers of oxidative stress, were significantly (both $p < 0.05$) higher in T2DM (respectively $10.1 \pm 1.1$ nmol/l and $9.7 \pm 2.5$ ng/ml) than in control (respectively $5.8 \pm 0.5$ nmol/l and $1.9 \pm 0.4$ ng/ml) islets. The degree of glucose-stimulated insulin release impairment was significantly correlated with the concentration of nitrotyrosine ($r = 0.96$, $p < 0.01$) and 8-OHDG ($r = 0.67$, $p < 0.05$).
**Conclusion:** These results provide direct evidence that in islets of T2DM patients the defect of insulin secretion is more marked in response to glucose than to other secretagogues, and is accompanied by altered expression of enzymes of the glycolytic pathway. The increased oxidative stress that was observed in T2DM islets can explain, at least in part, the defect of insulin release.

# OP 12

# Complications: role of endothelium

## 67

**Vascular effects of rosiglitazone in relation to its fluid-retaining properties**

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**Background and aims:** The exact mechanism of thiazolidinedione (TZD)-induced fluid retention is not known. In general, the initial trigger in fluid retention originates either in the kidney, the heart or the peripheral circulation (vasodilation, vascular leakage). A primary renal mechanism is unlikely because TZDs lower blood pressure (BP). Since TZD-induced fluid retention occurs more frequently in insulin-treated patients, and because insulin has obvious vasodilator properties, which are blunted in insulin resistance, we hypothesized that improvement of the vasodilator response to insulin is the key mechanism of TZD-related fluid retention. Therefore, our aim was to investigate whether rosiglitazone (RSG) can restore a blunted vascular response to hyperinsulinaemia and to determine whether such a change was paralleled by an increase in NO-dependent vasodilation.
**Materials and methods:** A randomized, double-blind, placebo-controlled, cross-over study was performed in 18 obese, non-diabetic subjects with two or more features of the Metabolic Syndrome (mean ± SD; age 46.1 ± 8.9 yr, BMI 31.8 ± 2.6 kg/m$^2$, BP 134 ± 10 /93 ± 5 mmHg). We investigated the effects of 12 weeks of RSG 4 mg bd or placebo on glucose disposal (insulin dose 120 mU/m$^2$/min), BP and forearm blood flow (FBF) at baseline, and during a hyperinsulinaemic euglycaemic clamp procedure (120 min). At the end of the insulin infusion, we assessed the vasoconstrictor response to the NO synthase inhibitor L-NMMA (0.4 mg/dL/min into the brachial artery). Furthermore, plasma volume (PV) and vascular leakage (assessed by transcapillary escape rate of $^{125}$I-albumin, TERalb), haematocrit and Atrial Natriuretic Peptide (ANP) were measured.
**Results:** (mean ± SE or 95% CI): As compared with placebo, RSG increased glucose disposal by 5.26 (1.68, 8.83)μmol/kg/min ($P = 0.007$). Hyperinsulinaemia (≈ 1700 pmol/L) did not increase FBF in either group, consistent with vascular insulin resistance (RSG treatment effect −8.2% [−27.2, 8,0] $P = 0.318$). Infusion of L-NMMA into the brachial artery, equally reduced FBF during both treatment periods (RSG: -21.4 ± 4.4%, vs placebo: −24.7 ± 2.9%, $P = 0.58$). RSG decreased diastolic BP by 5 mmHg (2.35, 6.87) ($P = 0.0005$). PV and ANP increased by 258 mL/1.73 m$^2$ (83, 433) ($P = 0.007$) and 12.1 pg/ml (0.7, 23.4) ($P = 0.039$) respectively, whereas haematocrit decreased by 0.019 (0.0082, 0.0297) ($P = 0.002$), all pointing towards an increased circulating volume. There was no treatment effect on vascular leakage (TERalb: placebo: 9.14 ± 0.52 vs RSG: 9.40 ± 0.51%/h). One subject developed significant oedema during RSG treatment (increase: weight and PV of 3.7 kg and 740 ml, respectively). RSG-induced changes in this subject were comparable to the mean response of the whole group.
**Conclusion:** RSG clearly improved the metabolic effect of insulin, reduced BP and increased PV. RSG did not restore the blunted vasodilator effects of insulin in this group of non-diabetic subjects with the Metabolic Syndrome, did not affect the contribution of NO to vascular tone during hyperinsulinaemia, and did not affect parameters of vascular leak. As such, these results provide no support for the view that TZDs increase transcapillary leakage of fluid as a result of either the augmentation of the NO-mediated vasodilator response to insulin or increase of capillary permeability.

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**C-reactive protein (CRP) enhances lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) expression in human aortic endothelial cells: relevance of LOX-1 to CRP-induced endothelial dysfunction**

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**Background and aims:** C-reactive protein (CRP), a characteristic inflammatory marker, is a powerful predictor of cardiovascular events. Recent data suggest that CRP may also promote atherogenesis through inducing endothelial dysfunction. Lectin-like oxidized low-density lipoprotein (oxLDL) receptor-1 (LOX-1) is a newly identified endothelial receptor for oxLDL that plays a pivotal role in oxLDL-induced endothelial dysfunction. Whether CRP may regulate endothelial LOX-1 and induce endothelial dysfunction through this receptor is unknown. The aim of this study was to determine the *in vitro* effect of CRP on LOX-1 expression in human aortic endothelial cells (HAECs) and the role of LOX-1 in CRP-induced human monocyte adhesion to endothelium and oxLDL uptake by endothelial cells.
**Materials and methods:** HAECs were incubated with CRP (1–25 μ g/ml) for 3 to 48 hours. In some experiments, HAECs were preincubated with antibodies against endothelin-1 (ET-1), interleukin-6 (IL-6), CD32 and/or CD64 prior exposure to CRP. At the end of the incubation period, LOX-1 gene and protein expression were measured by PCR and western-blot analysis, respectively. Monocyte adhesion to endothelial cells was determined by measuring monocyte myeloperoxidase (MPO) activity, and DiI-labeled oxLDL uptake by endothelial cells was assessed by fluorescence microscopy.
**Results:** Incubation of HAECs with CRP enhanced, in a dose- and time-dependent manner, LOX-1 mRNA and protein levels. Induction of LOX-1 protein was already present at 5 μ g/ml CRP and reached a maximum at 25 μ g/ml (LOX-1 protein expression (% of control values): controls: 100 ± 1; 5 μ g/ml CRP: 143 ± 25; 10 μ g/ml CRP: 145 ± 18; 25 μ g/ml CRP: 178 ± 8, $P < 0.05$). This effect was reduced by antibodies against CD32/CD64, ET-1 and IL-6 (LOX-1 protein expression (% of control values): controls: 100 ± 1; CRP: 178 ± 8, $P < 0.001$; CRP+anti-CD32: 127 ± 10, $P < 0.05$; CRP+anti-CD64: 145 ± 7, $P < 0.01$; CRP+anti-CD32+anti-CD64: 117 ± 13; CRP+anti-IL-6: 120 ± 19; CRP+anti-ET-1: 142 ± 22; CRP+anti-IL-6+anti-ET-1: 88 ± 6). The extent of stimulation of LOX-1 achieved by CRP was comparable to that elicited by high glucose and IL-6 and remained unchanged in presence of these factors (LOX-1 protein expression (% of control values): controls: 100 ± 1; CRP: 178 ± 8, $P < 0.001$; glucose: 209 ± 13, $P < 0.001$; CRP+glucose: 161 ± 5, $P < 0.01$; IL-6: 168 ± 15, $P < 0.01$; IL-6+CRP: 169 ± 12, $P < 0.01$). Finally, CRP increased, through LOX-1, both human monocyte adhesion to endothelial cells and oxLDL uptake by these cells.
**Conclusion:** These results demonstrate that CRP enhances endothelial LOX-1 expression and suggest a new mechanism by which CRP may promote endothelial dysfunction, that of inducing LOX-1.

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**Glucagon-like peptide-1 improves endothelial function in Type 2 diabetic patients**

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**Background and aims:** Glucagon-like peptide-1 (GLP-1) stimulates insulin secretion, inhibits glucagon secretion, delays gastric emptying and inhibits small bowel motility, all actions that contribute to the antidiabetogenic effect of GLP-1. There are some reports showing vascular respons from GLP-1. Whether GLP-1 receptors are expressed in endothelial cells is not known. We have in this study investigated whether GLP-1 improves the endothelial dysfunction in diabetic patients with an established coronary heart disease and whether such an effect correlates to an enhanced insulin sensitivity.
**Materials and methods:** Twelve male type 2 diabetic patients with established coronary heart disease and ten unmatched healthy subjects underwent infusion of GLP-1 or saline. Insulin sensitivity was measured by euglycemic, hyperinsulinemic clamp technique. Stimulated endothelial nitric oxide production was measured by post-ischemic flow-mediated vascular dilatation (FMD) of the brachial artery using ultrasonography. Human coronary endothelial cells were cultured and harvested pending analysis with Western blot.
**Results:** Western blots confirmed that GLP-1 receptors were expressed in human coronary endothelial cells. Furthermore, GLP-1 infusion significantly increased FMD in type 2 diabetic patients (3.1 ± 1.4 *vs.* 6.6 ± 1.5%, $P$=0.03) without any effect in healhty subjects (11.9 ± 4.5 *vs.*11.4 ± 3.2%, $P$=0.64). C-peptide levels were increased in type 2 diabetic patients (0.30 ± 0.2 *vs.* 0.86 ± 0.5 nmol/L, $P$=0.02) as well as in healhty subjects (0.65 ± 0.2 *vs.* 1.96 ± 0.8 nmol/L, $P$=0.01) and glucagon levels decreased by GLP-1 infusion in both groups. In contrast, whole body glucose uptake rates were not affected by GLP-1 infusion in type 2 diabetic patients (21 ± 8 *vs.* 23 ± 8 μmol·kg$^{-1}$·min$^{-1}$, $P$=0.41) nor in healhty subjects (66 ± 17 *vs.* 74 ± 11 μmol·kg$^{-1}$·min$^{-1}$, $P$=0.52).
**Conclusion:** GLP-1 receptors are expressed in human coronary endothelial cells. Infusion of GLP-1 improves endothelial function in type 2 diabetic patients with established coronary artery disease, without affecting glucose uptake. This positive vascular effect of GLP-1 adds yet another beneficial property of the peptide, increasing its clinical utility in type 2 diabetes in which endothelial dysfunction is a prominent feature.

## 70

**Protective effects of metformin on the endothelial dysfunction of the renal circulation induced by acute hyperglycemia in non-diabetic rabbits**

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**Background and aims:** Previous studies showed that high glucose levels, in the range observed in patients with type 2 diabetes, induce acute endothelial dysfunction in the rabbit renal circulation. Most population studies show the great impact of diabetes mellitus and its chronic complications on mortality and morbidity. Diabetic nephropathy is becoming the leading cause of end-stage renal disease (ESRD) in the industrialized countries. Some studies showed that almost 35% of all new admissions for renal replacement therapy were due to diabetes mellitus and most of these patients had diabetes type 2. The UKPDS study showed that overweight patients treated with metformin, compared with the conventional treatment group, had significant risk reductions for any diabetes-related endpoint, diabetes-related death as well as for all-cause mortality. The main purpose of the present study was to investigate the protective effects of metformin on the endothelial dysfunction of the renal circulation of non-diabetic rabbits acutely induced by levels of glucose usually observed in diabetic patients in daily clinical practice.
**Material and methods:** Isolated perfused kidneys from non-diabetic rabbits were acutely exposed (3 h) to normal (5.5 mM -control group) or high (15 mM) D-glucose. Glucose levels used in the present study correspond 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). Metformin (100 μM) was continuously infused into the renal circulation simultaneously with the infusion of high glucose solutions.
**Results:** Kidneys perfused with high glucose (15 mM) had endothelium-dependent (ACh-induced) maximal vasodilation blunted in comparison to control group (respectively 25 ± 4% and 41 ± 4%; n=7, P<0.01). Three-hour infusions of metformin restored the vasodilating effect of ACh in the renal circulation in the presence of high glucose, reaching the maximum of 43 ± 2% (n=7, P> 0.05 vs. control group). Metformin did not affect maximum vasodilation induced by ACh in the presence of normal glucose levels.
**Conclusions:** Acute hyperglycemia corresponding to the range observed in patients with type 2 diabetes induces endothelial dysfunction in the renal circulation of normal rabbits. Acute treatment with Metformin was able to protect the renal circulation against the effects of high glucose without affecting maximum vasodilation observed in the presence of normal glucose.
*Supported by: Merck*

## 71

**Beneficial effects of rosiglitazone on insulin resistance and endothelial dysfunction in patients with Type 2 diabetes**

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**Background and aims:** Alterations in endothelial function have been widely demonstrated in patients with type 2 diabetes. Some experimental data suggest that the insulin sensitizer rosiglitazone (RSG), a potent selective peroxisome proliferator-activator receptor gamma (PPARγ) agonist may improve the endothelial function. The aim of the trial was to investigate the effects of RSG on several markers of endothelial dysfunction and insulin resistance.
**Materials and methods:** This was a multicenter, randomized, double-blind, placebo (PBO)-controlled, parallel-group study that consisted of a screening visit, a 4-week single-blind PBO run-in period, and a 12-week double-blind treatment period. where patients were receiving RSG 8 mg (48 patients) or PBO (54 patients) once daily. Adhesion molecules such as E-selectin and vascular cell adhesion molecule-1 (VCAM-1), and other soluble markers such as the plasminogen activator inhibitor (PAI-1)-antigen, the tissue plasminogen activator (tPA)-antigen and the von Willebrand factor antigen were measured in sera from type 2 diabetic patients. For adhesion molecules, results were compared to those from 100 healthy controls. Free fatty acids were measured at fasting and until 240 min after a standard meal (AUC -FFA).
**Results:** In diabetic patients E-selectin and VCAM-1 baseline levels were higher (76 ± 6 ng/ml vs 48.8 ± 1.8 ng/ml, p < 0.0001 and 472.4 ± 21.5 ng/ml vs 388.6 ± 6.3 ng/ml, NS, respectively), when compared to the control group. E-selectin baseline levels were significantly correlated to fasting glycemia, fructosamine, triglycerides, C-peptide, alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT), PAI-1, tPA-antigen (p = 0.05 to 0.0001). VCAM-1 baseline levels were significantly correlated to fructosamine, ALAT and ASAT, and to von Willebrand factor antigen (p = 0.04 to 0.0001). As compared to PBO, patients who received RSG showed a significant decrease (expressed as delta) of E-selectin, PAI-1, tPA-antigen and von Willebrand factor antigen levels (p=0.004, for the 4 parameters), as well as a significant decrease in fructosamine, insulin levels, AUC-FFA and in diastolic blood pressure, whereas changes in VCAM-1 levels did not differ significantly from those of the PBO group. The decrease of these 4 markers of endothelial dysfunction did not correlate with the changes in glycemic parameters. Changes of E-selectin and in AUC-FFA showed a positive and significant correlation (r=0.25, p=0.03).
**Conclusion:** In type 2 diabetes, these data are consistent with a known burden effect of insulin resistance on the endothelial dysfunction and strongly suggest a beneficial effect of RSG on endothelial function, possibly through a decrease in free fatty acids.

## 72

**Interaction of insulin and IGF-I system in human coronary artery endothelial cells**

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**Background and aims:** Coronary heart disease is a prevalent cause of morbidity and mortality in diabetes. Low circulating IGF-I is associated with vascular disease. Little is known about the function of the IGF-I system in human coronary artery endothelial cells (HCAEC). We have studied the gene expression of IR (insulin receptor), IGF-IR (insulin-like growth factor-I receptor), IGF-I (insulin-like growth factor-I), GHR (growth hormone receptor) and IGFBP-4 (insulin-like growth factor binding protein-1); the activation of IGF-IR and IR and the presence of hybrid IR/IGF-IR in HCAEC.
**Materials and methods:** HCAEC provided by Clonetics® were cultured according to manufactures instructions in growth medium with serum and then starved for 24 hours before the experiments. Gene expression of polypeptides was measured quantitatively by real-time PCR and normalised to GAPDH. Activation of IGF-IR and IR β-subunit and the presence of hybrid IR/IGF-IR were analyzed by immunoprecipitation and Western blot.
**Results:** The relative expression compared to IGFBP-4 (100%) was for IGF-IR 15.6% (p<0.001), for IGF-I 4.6% (p<0.001), for IR 1.9% (p<0.001) and for GHR 0.07% (p<0.001). Growth medium down regulated the gene expression of IR with 256.6% (p=0.03) and of IGF-I with 1082.2% (p<0.001). Activation of the IGF-IR β-subunit was obtained by IGF-I $10^{-1}$%$10^{-8}$M, insulin $10^{-6}$M, but not by insulin $10^{-10}$-$10^{-9}$M. The IR β-subunit was phosphorylated by insulin $10^{-9}$-$10^{-8}$M and IGF-I $10^{-9}$-$10^{-8}$M. After immunoprecipitating with specific IGF-IR or IR α-subunit antibodies and subsequently blotting the gels with appropriate anti-IGF-IR or anti-IR β-subunit antibodies we detected bands for β-subunits of both IR and IGF-IR indicating the presence of hybrid IR/IGF-IR.
**Conclusion:** Our study provides experimental evidence for a role of IGF-IR and possibly hybrid IR/IGF-IR in human coronary artery endothelial cells.

# OP 13

# EASD/ESC Session: diabetes and the heart

## 73

**Impaired migratory capacity of endothelial progenitor cells in hyperglycemia**

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**Background:** Circulating endothelial progenitor cells (EPCs) originating from the bone marrow are thought to be involved in the maintenance of an intact endothelial layer. Cardiovascular risk factors like diabetes mellitus (DM) have been shown to contribute to the development of endothelial dysfunction, a process which might partially be mediated by a decrease in EPC number and function. The exact pathomechanisms leading to the impairment of EPC function in DM have, however, not been clarified yet. Therefore, in the present study the impact of hyperglycemia (HG), a main feature of DM, on the functional characteristics of EPCs was investigated.
**Methods:** Mononuclear cells (MNCs) of healthy donors (n=8) were cultivated under HG conditions (12 mM D-Glucose) or in osmotic control medium (Con; 5 mM D-Glucose plus 7 mM L-Glucose) for 7 days. EPC adhesion to activated human coronary artery endothelial cells and integration into endothelial structures (Matrigel assay) were assessed. Furthermore, mRNA expression of the matrix metalloproteinase 9 (MMP9) and its specific inhibitor TIMP1 were assessed by quantitative realtime RT-PCR and MMP9 activity was analyzed using gelatin zymography.
**Results:** A significant reduction in the capability to incorporate into a network of endothelial cells was observed under HG (HG: 14.9 ± 1.8% vs. Con: 27.1 ± 3.6%; p=0.005), accompanied by a diminished MMP9 mRNA expression (HG: 2.37 ± 0.64 vs. Con: 5.23 ± 1.51; p<0.05) and activity (0.42 ± 0.11 vs. 0.64 ± 0.14; HG vs. Con; p<0.04) as well as an increased mRNA expression of the MMP9 inhibitor TIMP1 (HG: 1.16 ± 0.16 vs. Con: 0.84 ± 0.15; p<0.05). However, no difference was seen in the ability of EPC to adhere to activated endothelial cells (HG: 14.9 ± 2.0 vs. Con: 13.7 ± 2.0 cells per field; p=0.66).
**Conclusion:** HG, a main feature of DM, affects important functional characteristics of EPCs, such as the ability to invade a matrigel and incorporate into endothelial cell structures. These results may provide further insight into the pathomechanisms of macro- and microvacular endothelial dysfunction in DM.

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**Suppression of rage as a basis of simvastatin-dependent plaque stabilization in Type 2 diabetes**

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The clinical benefits of statins in diabetes are attributed to changes in plaque composition leading to reduced metalloproteinase (MMP) activity and plaque stabilization. However, the molecular mechanism(s) underlying this effect are not completely elucitated. Strong evidences suggest a central role for RAGE (receptor for advanced glycation end products [AGEs]) in the accelerated progression of atherosclerosis observed in diabetes. In particular, we have recently demonstrated enhanced expression of RAGE in human diabetic plaques, and provided evidence that it is associated with inducible cyclooxygenase and PGE synthase-1 (COX-2/mPGES-1) overexpression, and PGE2-dependent MMP generation leading to plaque rupture. Thus, the aim of this study was to characterize the effect of simvastatin on the inflammatory infiltration and the expression of RAGE and RAGE-dependent plaque-destabilizing genes in human carotid plaques. Seventy type 2 diabetic patients with asymptomatic carotid artery stenosis (>70%) were randomized to American Heart Association step 1 diet plus simvastatin (40 mg/d) or American Heart Association step 1 diet alone for 4 months before endarterectomy. Plaques were subjected to analysis of RAGE, COX-2, mPGES-1, MMP-2 and MMP-9, lipid and oxidized LDL (oxLDL) content, and collagen content by immunocytochemistry, Western blot and RT-PCR, whereas zymography was used to detect MMP activity. Immunocytochemistry was also used to identify CD68+ macrophages, CD3+ T-lymphocytes, smooth muscle cells (SMCs) and HLA-DR+ inflammatory cells.
Plaques from simvastatin group had fewer (P<0.0001) macrophages (7 ± 2% vs 23 ± 6%), T-lymphocytes (13 ± 4 vs 72 ± 9 cells/mm$^2$), and HLA-DR+ cells (9 ± 3% vs 25 ± 7%); less (P<0.0001) immunoreactivity for RAGE (9 ± 2% vs 25 ± 5%), COX-2 (11 ± 3% vs 26 ± 4%), mPGES-1 (5 ± 1% vs 22 ± 4%), MMP-2 (8 ± 3% vs 24 ± 4%), and MMP-9 (10 ± 3% vs 26 ± 6%); reduced (P<0.0001) gelatinolytic activity; increased (P<0.0001) collagen content (31 ± 5% vs 12 ± 4%), and reduced (P<0.0001) lipid and oxLDL content (6 ± 2% vs 21 ± 4%). Interestingly, inhibition of RAGE expression by simvastatin was observed not only in plaque sections but also in plaque-derived macrophages.
In conclusion, this study demonstrates that simvastatin decreases inflammation and inhibits RAGE expression in plaque macrophages, and this effect in turn may contribute to human plaque stabilization by inhibition of PGE2-dependent biosynthesis of metalloproteinases responsible for plaque rupture.

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**Local epicardial adipose tissue inflammation is associated with serum insulin and insulin resistance in patients with advanced coronary artery disease**

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**Background:** Vascular and extravascular expression of inflammatory mediators may adversely influence coronary lesion formation and plaque stability. Although omental adipose tissue is a recognized source of inflammatory cytokines in patients with insulin resistance, the importance of other fat depots in regard to perivascular inflammation has not been elucidated. Accordingly, we examined the properties of epicardial adipose tissue in patients undergoing surgical coronary revascularisation.
**Methods:** Paired samples of epicardial (epi-fat) and subcutaneous (sc-fat) adipose tissue were harvested during elective CABG surgery (n=42; age 65 ± 10; 57% history of diabetes). Tissue expression of chemokine (MCP-1) and inflammatory cytokines (IL-6, IL-12, TNF-α) was analyzed by real time RT-PCR (mRNA), and ELISA (protein release over 3 hours normalized by total protein release). Local inflammatory markers expression associacions with categorical (ie, gender, smoking, hypertension, diabetes, history of acute coronary syndrome, extent of CAD, treatment) and continous variables (ie, age, body mass index, serum glucose, lipids, serum insulin, insulin resistance calculated as homeostasis model assessment, HOMA-IR) were further analyzed.
**Results:** Higher expression of MCP-1 (p<0.01), IL-6 (p<0.001), IL-12 (p<0.05), and TNF-α (p<0.05) was observed in epi-fat, as compared with sc-fat on mRNA (target mRNA/GAPDH) and protein levels. Mean value of serum insulin and HOMA-IR were 5.1 ± 0.8 mU/L and 1.7 ± 0.3, respectively. As expected, there was significant correlation (Spearman) observed between BMI and serum insulin and HOMA-IR (r=0.45, P<0.05; r=0.43, P<0.05, respectively). No significant associations were detected between epicardial adipose tissue inflammation and categorical variables. Serum insulin and HOMA-IR were not predictive of local inflammatory burden in sc-fat, wheras we found significant correlations with inflammatory biomarkers released by epi-fat. Serum insulin significantly correlated with epi-fat IL-6 (r=0.64, P<0.001), and epi-fat IL-1b (r=0.58, P<0.01). Similarly, HOMA-IR significantly correlated with epi-fat IL-6 (r=0.64, P<0.001), and epi-fat IL-1b (r=0.64, P<0.001).
**Conclusions:** 1. Epicardial (pericoronary) adipose tissue may contribute to a low-grade inflammation in high risk cardiac patients; 2. Coronary arteries are exposed to inflammatory signals from the perivascular tissue; 3. Local inflammatory burden in adipose tissue surrounding coronary arteries is associated with serum insulin and insulin resistance in patients with advanced coronary artery disease.

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**CRP elevation in diabetic patients with acute myocardial infarction – The Munich Myocardial Infarction Registry**

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**Background and aims:** C-reactive-protein (CRP) is a marker for subclinical inflammation, which contributes to the pathogenesis of atherosclerosis. There is evidence that CRP is elevated in patients with diabetes. The aim of

the study was to assess CRP in patients with acute myocardial infarction (AMI) and to compare the results between diabetic (D) and non-diabetic (ND) patients.
**Materials and methods:** 1237 patients with AMI (D n = 479 (38,7%); ND n = 758 (61,3%)) who were admitted to the coronary care unit between 1999 and 2003 were assessed. Patients were studied within the Munich Myocardial Infarction Registry. Plasma CRP was analysed with a standard assay.
**Results:** CRP was significantly higher in D than in ND with acute myocardial infarction (36 ± 61 vs. 28 ± 56 mg/l, p=0,001). Diabetic patients, who died in the hospital (n= 102, 21,3%), demonstrated higher CRP-levels than those who survived (54 ± 67 vs. 31 mg/l ± 58 mg/l; p<0,001). Mortality within the first 24 hours after admission was also accompanied with elevated CRP levels (58 ± 80 vs. 35 ± 59 mg/l; p<0,05). 35% (n= 163) of D did not report angina pectoris. These patients exhibited a nearly twofold higher CRP compared to those, who presented with angina pectoris (51 ± 74 vs. 26 ± 50 mg/l; p<0,001).
Diabetic patients with a CRP equal or higher than the median value (8 mg/dl) demonstrated a higher HbA1 c (7,7 ± 1,7 vs. 7,1 ± 1,5%; p<0,01), creatinine (1,7 ± 1,1 vs. 1,2 ± 0,6 mg/dl; p<0,001) and white blood cell count (12.900 ± 5.300 vs. 10.900 ± 4.000/µl; p<0,001) compared to those without. The presence of co-morbidities was associated with increase in CRP: renal failure (47 ± 67 mg/l vs. 26 ± 54 mg/l; p<0,001), peripheral artery disease (44 ± 64 mg/l vs. 34 ± 62 mg/l; p=0,01) and previous stroke (47 ± 71 mg/l vs. 34 ± 59 mg/l; p<0,05).
**Conclusion:** In diabetic patients with AMI, CRP is not only higher than in non-diabetic patients, but it is also related to silent myocardial ischemia, co-morbidity and mortality. The need for assessment of CRP and subsequent earliest diagnostic procedures and interventions is emphasized.

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**N-terminal pro B-Type natriuretic peptide and long-term mortality in stable coronary heart disease: relation to diabetes status**

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**Background and aims:** Patients with diabetes have an increased risk of cardiovascular disease. N-terminal pro brain natriuretic peptide (NT-proBNP) is associated with left ventricular dysfunction and inducible ischaemia and may be an additive risk marker in patients with diabetes and chronic coronary heart disease. We therefore assessed the relationship between NT-proBNP levels related to diabetes status and long-term, all-cause mortality in a large cohort of patients with stable coronary heart disease.
**Materials and methods:** For the purpose of this study we measured NT-proBNP in baseline samples from 1022 patients (196 diabetics and 826 non-diabetics) referred for angiography in1990–1992 because of symptoms or signs of coronary heart disease. All-cause mortality was determined after a median follow-up of 9 years, and patients were stratified in 4 groups according to NT-proBNP above or below the median and diabetes status.
**Results:** At follow-up 285 patients had died [204 non-diabetics (25%) and 81 diabetics (41%)]. Median (inter-quartile range) NT-proBNP was significantly lower in non-diabetics than in diabetics [162 pg/ml (61-427) vs. 231pg/ml (76-633), p=0.02]. Patients with diabetes and supramedian NT-proBNP were older, had a lower left ventricular ejection fraction, lower creatinine clearance and a higher prevalence of previous myocardial infarction and 3-vessel disease compared to non-diabetics with NT-proBNP below the median. The unadjusted hazard ratios of the four different groups are shown in the table. In a multivariate Cox regression model diabetics with NT-proBNP below the median had comparable risk to non-diabetic patients with supramedian NT-proBNP. The combination of NT-proBNP and diabetes substantially improved risk stratification beyond that provided by either alone (see table) and added prognostic information beyond age, gender, family history of ischemic heart disease, previous myocardial infarction, angina, hypertension, smoking, creatinine clearance, lipids, left ventricular ejection fraction and coronary disease at angiography.
**Conclusion:** NT-proBNP and diabetes are complementary independent risk factors of long-term mortality in patients with stable coronary heart disease. Combined assessment provides risk stratification superior to that provided by either alone.

Table 1

| | Univariate HR (95% CI) | p-value | Multivariate HR (95% CI) | p-value |
|---|---|---|---|---|
| Low NT-proBNP/ No diabetes | 1.0 | | 1.0 | |
| Low NT-proBNP/ Diabetes | 1.9 (1.1–1.3) | 0.02 | 1.5 (0.9–2.6) | ns |
| High NT-proBNP/ No diabetes | 3.1 (2.4–4.1) | <0.0001 | 1.8 (1.3–2.5) | 0.001 |
| High NT-proBNP/ Diabetes | 5.5 (3.8–8.0) | <0.0001 | 3.0 (2.0–4.5) | <0.0001 |

## 78

**Insulin increases protein expression of the hypoxia inducible factor 1-alpha in vascular smooth muscle cells: a phenomenon potentially involved in post-ischemic neovascularization that is attenuated in insulin resistance**

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**Background and aims**: Hypoxia-inducible factor 1 (HIF-1) is a transcription factor involved in physiological and pathological angiogenesis -in particular via induction of Vascular Endothelial Growth Factor (VEGF) synthesis- and in erythropoiesis. It consists of two subunits, HIF-1 α which is inducible and HIF-1 β which is constitutively expressed. The term "HIF" derives from the ability of hypoxia to stimulate this factor, which is deeply involved the adaptative response to hypoxia in terms of neo-angiogenesis and hemoglobin transport. A non-hypoxic activation, however, has been described. We previously demonstrated that insulin stimulates VEGF synthesis and secretion via PI3-K/Akt and MAPK pathways in vascular smooth muscle cells (VSMC) from humans and insulin sensitive lean Zucker (fa/+) rats and that this effect is attenuated in the insulin resistant obese Zucker (fa/fa) rats. It is not known whether these insulin effects involve an insulin-induced activation of HIF-1α. Aim of this study is to evaluate whether insulin induces HIF-1α protein expression in VSMC, to clarify the signalling pathways involved and the role of insulin-resistance.
**Materials and methods:** We incubated cultured aortic VSMC derived from humans and from insulin sensitive (fa/+) and resistant (fa/fa) Zucker rats with 2nmol/l insulin for 6 hours and we measured HIF-1α protein expression (western blot) in the presence and in the absence of the PI3-K inhibitor LY294002 (100 µmol/l) and of the MAPK inhibitor PD98059 (30 µmol/l). Data are evaluated by a densitometric analysis of western blots and expressed as percent of baseline (mean±SEM).
**Results:** In human aortic VSMC, HIF-1α protein expression after a 6-hour incubation with 2 nmol/l insulin was 268.01 ± 1.67% of baseline (n=4, p=0.0001). This increase was blunted by PI3-K inhibition and MAPK inhibition. Similarly, in aortic VSMC from Zucker fa/+ rats HIF-1α protein expression after a 6-hour incubation with 2 nmol/l insulin was 185.9 ± 1.12% of baseline (n=4, p=0.0001): also in this case, PI3-K and MAPK inhibition blunted the insulin-induced expression of HIF-1α . In VSMC from the insulin resistant Zucker fa/fa rats the insulin-induced increase of HIF-1α protein expression -although significant (n=4, p=0.0001)- was lower than in VSMC from Zucker fa/+ rats (112 ± 1.34% vs 185.9 ± 1.12%, p=0.0001). As far as the concentration-dependence of the insulin effect in Zucker fa/+ rats is concerned, also insulin concentrations of 0.5 and 1 nmol/l increased HIF-1α (n=4, p 0.002 and p=0.0001, respectively).
**Conclusion:** i) insulin increases HIF-1 α protein expression in aortic VSMC from humans and insulin sensitive Zucker fa/+ rats, with a mechanism involving both PI-3K and MAPK pathways; ii)this insulin effect is deeply reduced in VSMC from insulin resistant Zucker fa/fa rats. Thus, in VSMC HIF-1 α protein expression is elicited by insulin with a non-hypoxic mechanism, and could contribute to neovascularization, likely via the VEGF increase that we previously demonstrated: the impairment of the insulin/HIFα/VEGF pathway in VSMC from insulin resistant animals could account for the reduced post-ischemic collateral vessel formation observed in the insulin resistant states.
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# OP 14

# Insulin delivery in Type 1 diabetes

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**Insulin pump therapy in children with diabetes: safety, efficacy and insulin adjustment**

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**Background and aims:** Many trials have shown that continuous subcutaneous insulin infusion (CSII) reduces provides better glycemic control. However its safe administration in children and adolescents is limited because of age-related developmental and cognitive issues.To evaluate the safety and efficacy of CSII method and insulin requirement in patients with type 1 diabetes in different age groups.
**Materials and methods:** During the period of 4 months we analysed data of 100 randomly chosen patients with type 1 diabetes collected during their scheduled visits in outpatient clinic; the mean age was 10.2 years (1.6 to 18 years), mean duration of diabetes 4.57 years (0.6 to 16 years) and mean duration of CSII therapy 1.75 years (0.5 to 3.0 years). We have analysed the data from insulin pumps memory, transmitted to PC computer with software Pumps & Meters program. During visits HbA1 c, patient weight and height was measured.
**Results:** The mean total daily dose of insulin was 0.79 ± 0.02 units/kg (0.3 to 2.0 units/kg/day). Basal insulin constitute on average 35,6 ± 1.1% of daily dose (5% to 70%). There were statistically significant higher contributions of basal insulin dose in patients who do not use insulin boluses with meals and was significantly lower in prepubertal children ($p<0.05$). Around 3% of patients made mistakes in programming of the basal insulin and 10% of patients did not use meals boluses. In one case an episode of severe hypoglycaemia was recorded and there was excessive insulin dosage in daily boluses (156 units/day). The mean HbA1 c values evaluated on the day of the visit was 7.63 ± 0.09% (5.15 to 12.5%) and those values positively correlated with patient BMI, higher contribution of basal insulin in total daily dose and in patients group forgetting meal bolus HbA1 c was higher: 8.67 ± 0.57% ($p<0,05$). Statistically significant better metabolic control was recorded among children less than 10 years of age.
**Conclusion:** CSII may be safely and efficiently used in children with type 1 diabetes in different age groups. This method of treatment requires regular visits in outpatient clinic, proper education concerning that type of insulin therapy and the frequent controlling of data which was registered in pump memory.

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**Treatment study in clinical practice to evaluate the effect of long-term treatment with insulin glargine in children and adolescents with Type 1 diabetes**

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**Background and aims:** Tight glucose control is especially difficult to achieve in children and adolescents due to compounding endocrine, behavioural and social factors. Insulin glargine (LANTUS®; GLAR) is the only basal long-acting insulin analogue to provide 24-hour basal glucose control with a once-daily dose. This ongoing, open study of clinical practice compares insulin glargine with NPH insulin or semilente (NPH/semi) in terms of glycaemic control ($HbA_{1c}$), quality of life (QoL) and hypoglycaemia in paediatric patients with Type 1 diabetes.
**Materials and methods:** Patients received prandial insulin (regular or lispro) and either once daily (6 PM-12 AM) GLAR (n=74), titrated to a target fasting blood glucose (FBG) of 4.4-7.8 mmol/L [80–140 mg/dL], or NPH/semi (NPH administered once-, twice- or three-times daily; semi administered once daily at bedtime) (n=68). The NPH/semi treatment groups were titrated to a target FBG of 4.4-8.9 mmol/L [80–160 mg/dL] (a wider range than the GLAR group to prevent nocturnal hypoglycaemia).
**Results:** From baseline to endpoint, the total insulin dose increased in both groups (0.90 ± 0.19 to 0.98 ± 0.24 IU/kg [$p=0.002$] vs 0.91 ± 0.31 to 0.98 ± 0.32 IU/kg [$p=0.008$]; GLAR versus NPH/semi); the basal insulin dose remained unchanged in the GLAR group (0.31 ± 0.09 to 0.31 ± 0.06 IU/kg) but decreased in the NPH/semi group (0.32 ± 0.11 to 0.27 ± 0.11 IU/kg; $p=0.01$); prandial insulin increased in both groups (GLAR: 0.60 ± 0.17 to 0.67 ± 0.20 IU/kg, $p=0.20$; NPH/semi: 0.59 ± 0.28 to 0.71 ± 0.30 IU/kg, $p=0.007$). Patients treated with NPH/semi for 27.7 ± 12.6 months had a significant increase in $HbA_{1c}$ levels (7.7 ± 1.6% to 8.1 ± 1.6%; $p=0.002$); GLAR treatment for 29.4 ± 11.6 months resulted in no significant increase (7.3 ± 1.0% to 7.5 ± 1.1%, [$p=0.06$]; treatment lengths differed as some patients were lost at follow-up). However, the incidence of symptomatic hypoglycaemia was comparable between the two groups at baseline and endpoint (2.11 vs 2.07 [GLAR] and 2.13 vs 2.08 [NPH/semi] episodes/week). The overall incidence of severe hypoglycaemia was significantly lower with GLAR compared with NPH/semi (0.15 vs 0.66 events/patient-year; $p=0.01$). Perceived QoL was better with GLAR.
**Conclusion:** This study shows that, despite stricter titration regimens, GLAR is associated with better glycaemic control, a lower incidence of severe hypoglycaemia and an improved QoL compared with NPH insulin and semilente, and can facilitate treating to target $HbA_{1c}$ in this difficult patient population with Type 1 diabetes.

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**Benefits of insulin detemir over NPH insulin in children and adolescents with Type 1 diabetes: Lower and more predictable fasting plasma glucose and lower risk of nocturnal hypoglycaemia**

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**Background and aims:** The soluble, basal insulin analogue, insulin detemir, has previously proved effective in obtaining glycaemic control with predictable glucose levels in adults with Type 1 diabetes on a basal-bolus regimen. The aim of this 26-week, multinational, open-label, randomised 2:1 (insulin detemir:NPH insulin), parallel group trial was to compare the effect of insulin detemir and NPH insulin in children and adolescents with Type 1 diabetes.
**Materials and methods:** Subjects received insulin detemir or NPH insulin once or twice daily (according to their pre-trial regimen), and pre-meal insulin aspart. A total of 347 (140 pre-pubertal and 207 pubertal) subjects (insulin detemir: 232, NPH insulin: 115) with baseline $HbA_{1c}$ 8.8 ± 1.2% [mean ± SD], age 11.9 ± 2.8 yrs, body mass index (BMI) 19.2 ± 2.8 kg/m$^2$ and duration of diabetes 5 yrs (range 1-15 yrs) received treatment.
**Results:** Mean $HbA_{1c}$ decreased by 0.8% for all subjects to 8.0%. Insulin detemir was non-inferior to NPH insulin with a mean difference (insulin detemir - NPH insulin) = 0.09 [95% C.I.: -0.12, 0.29]. Mean home-measured fasting plasma glucose with insulin detemir was lower than with NPH insulin (8.44 vs. 9.58 mmol/L, $p=0.022$), as was the within-subject variation in fasting plasma glucose (SD=3.32 vs. 4.29 mmol/L, $p<0.001$). The overall shape of the 8-point plasma glucose profiles as well as the mean nocturnal plasma glucose level were similar with insulin detemir and NPH insulin ($p=0.302$ and $p=0.194$, respectively). The overall risk of hypoglycaemia was similar with the two treatments ($p=0.351$), whereas the risk of nocturnal hypoglycaemia (22:00 - 07:00) was 36% lower with insulin detemir than with NPH insulin ($p=0.011$). Baseline-adjusted BMI was lower with insulin detemir (19.3 vs. 19.8 kg/m$^2$, $p<0.001$) after 26 weeks. The general safety profile of insulin detemir was similar to that of NPH insulin.
**Conclusion:** Basal-bolus therapy with insulin detemir or NPH insulin and pre-meal insulin aspart for 26 weeks in children and adolescents with type 1 diabetes improved $HbA_{1c}$ to a similar degree. The lower and more predictable fasting plasma glucose, lower risk of nocturnal hypoglycaemic episodes and a lower BMI observed with insulin detemir are clinically significant advances compared to NPH insulin.
*This study was sponsored by Novo Nordisk*

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**Comparison of continuous subcutaneous insulin infusion (CSII) vs. multiple daily insulin injections (MDI) in regard to metabolic control. Results of the 5-Nations trial**

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**Background and aims:** Continuous subcutaneous insulin infusion (CSII) is an alternative to multiple daily injections (MDI) as intensive insulin therapy for optimizing glycaemic control in type 1 diabetes. However, the value and place of CSII in the management of type 1 diabetes remains controversial. Previous randomized controlled trials (RCTs) comparing CSII to MDI mostly date from the 1980s using outdated insulin's and pump technology. The number of subjects was usually small and the duration of usage of each mode of insulin administration short. Rapid acting insulin analogues, now a standard part of MDI, have only been used in one crossover RCT published to date. Therefore, the goal of the study was to determine whether CSII differs from multiple daily insulin injection therapy (MDI) with respect to metabolic control in people with type 1 diabetes mellitus.
**Material and methods:** The 5 Nations trial was a randomized, controlled, crossover trial, running in 11 centers in 5 European countries. 272 patients with type 1 diabetes mellitus previously treated with MDI for at least 6 months were included and have been treated with CSII or MDI during a 2-month run-in period followed by a 6-month treatment period, respectively. As the primary endpoint the quality of metabolic control has been assessed by hemoglobin $A_{1c}$, and blood glucose fluctuations. The significant secondary metabolic efficacy endpoints were mean daily blood glucose values, determined from standardized 24-hour blood glucose profiles, and the frequency and severity of hypoglycemic episodes.
**Results:** CSII treatment resulted in lower $HbA_{1c}$ (7.45% vs. 7.67%, $p<0.001$), mean blood glucose level (8.6 ± 1.8 mmol/l vs. 9.4 ± 1.9 mmol/l, $p<0.001$) and caused less fluctuation in blood glucose levels than MDI (± 3.9 mmol/l on CSII compared to ± 4.3 mmol/l on MDI, $p<0.001$). There was a marked reduction in the frequency of significant hypoglycemic events using CSII compared to MDI, with an incidence ratio of 2.60 (95% CI: 2.08–3.25) and 2.61 (95% CI: 1.59–4.29) for any and severe hypoglycemia requiring assistance, respectively.
**Conclusion:** CSII usage offers significant benefits over MDI for individuals with type 1 diabetes with improvement in all significant metabolic parameters.
*Supported by: Disetronic Medical Systems AG*

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**Changing from prior premix insulin regimen to insulin glargine in patients with Type 1 diabetes: results from the AT.LANTUS trial**
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**Background and aims:** The use of premix insulin for the treatment of Type 1 diabetes is common in clinical practice. However, the introduction of insulin glargine (LANTUS®), a basal long-acting insulin analogue, offers patients a more physiological insulin replacement owing to its smooth time-action profile, no pronounced peaks and 24-hour duration of action. In combination with prandial insulin analogues, insulin glargine provides equivalent glycaemic control to continuous subcutaneous insulin infusion, the gold standard treatment for patients with Type 1 diabetes. This study was carried out to determine the optimal method for initiating and maintaining insulin glargine therapy in a large Type 1 patient population inadequately controlled on their previous regimen.
**Materials and methods:** This was a 24-week, multinational (n=59), multicentre (n=401), randomized trial carried out to compare two insulin glargine algorithms to achieve normoglycaemia (in terms of incidence of severe hypoglycaemia, change in metabolic control and insulin dose). The titration was based on a target fasting blood glucose (FBG) of 4.4–6.7 mmol/L (80–120 mg/dL). Analysis of the full patient population demonstrated no difference in endpoints between the two treatment algorithms (data not shown). The results of patients changing from sub-optimal premix insulin treatment to an insulin glargine-based regimen are reported in this abstract.
**Results:** A total of 2410 patients were treated, of which 2140 patients completed the study as per protocol. Of these patients, 122 were previously treated with a premix insulin regimen of ≤2 injections/day only and 71 were previously treated with ≥3 injections/day premix insulin regimen only. Patients were changed from premix insulin to once-daily insulin glargine + prandial insulin. There was a significant improvement in all endpoints for both groups (Table). When patients were switching from an initial premix-based regimen to insulin glargine plus prandial insulin, a 1% decrease in $HbA_{1c}$ was observed. Overall, the incidence of severe hypoglycaemia was 5.7% (algorithm 1: 4.1%; algorithm 2: 7.4%) with no significant difference between the two algorithms..
**Conclusion:** The results of this subanalysis demonstrate the clinical benefit of changing patients from premix insulin therapy to a more physiological insulin regimen, such as basal insulin glargine plus prandial insulin. In this population with poor control at baseline, the treatment algorithms allow better titration of insulin glargine and facilitate achieving better glycaemic control in a diverse patient population.

| | Prior premix | Baseline | Endpoint |
|---|---|---|---|
| $HbA_{1c}$, % | ≤2 injections/day | 9.2 | 8.0 |
| | ≥3 injections/day | 8.7 | 8.0 |
| Fasting blood glucose, mmol/L (mg/dL) | ≤2 injections/day | 9.5 (170.4) | 5.8 (104.8) |
| | ≥3 injections/day | 10.6 (191.2) | 7.1 (127.8) |
| Body weight, kg | ≤2 injections/day | 68.4 | 69.2 |
| | ≥3 injections/day | 70.8 | 72.1 |
| Insulin glargine dose, IU/day | ≤2 injections/day | 28.1 | 33.3 |
| | ≥3 injections/day | 30.2 | 36.6 |

*Supported by: Aventis Pharma.*

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**Exercise does not affect the absorption of insulin glargine in people with Type 1 diabetes mellitus**
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**Background and aims:** Basal insulin replacement as part of intensive insulin therapy should provide a predictable physiological supply of insulin over 24 hours, with the aim of achieving low blood glucose levels without causing hypoglycaemia. Achieving this aim is sufficiently challenging when people are at rest and is further complicated during exercise, which may modify both the insulin requirement and the absorption of exogenous insulin. In people with Type 1 diabetes, insulin glargine, a basal, long-acting insulin analogue, has been demonstrated to closely mimic the physiology of endogenous basal insulin concentrations observed in people without diabetes owing to its 24-hour duration of action after once-daily dosing. This study explored the effects of 30 min of intense exercise on the absorption of subcutaneously (sc) administered $^{125}$I-labelled insulin glargine (IGlarg) in people with Type 1 diabetes.
**Materials and methods:** A total of 13 people (12 male, 1 female) with Type 1 diabetes, mean (± SD) age 33.3 (± 6.5) years, BMI 26.8 (± 3.3) kg/m$^2$ and $HbA_{1c}$ 7.6 (± 1.3) % participated in the study. IGlarg, at the usual basal insulin dose (range: 14-46 IU), was injected sc into the thigh on the evening (9 pm) prior to the study day on two occasions 1 week apart. The next morning, patients were given their usual dose of rapid acting insulin (insulin aspart; insulin lispro) sc into the anterior abdominal wall prior to a standardised mixed meal (500 kcal). Patients were then randomly assigned to exercise (30-min exercise on an ergocycle at approx 55% $VO_2$ max) or no exercise 1 hour from commencement of the meal. Radioactivity counts at the site of injection were taken before (background) and immediately after injection of radiolabelled IGlarg and at 30-min intervals throughout the study day. Blood samples were collected for determination of plasma glucose and insulin concentrations. Area under the curve above fasting (ΔAUC) for glucose and insulin profiles were calculated by the trapezoidal method and compared by Wilcoxon's test.
**Results:** Linear regression analysis showed no statistically significant difference in the rate of disappearance of IGlarg (repeated measures ANOVA; $p=0.548$). No difference in ΔAUC insulin (mean ± SEM) was observed between the non-exercise versus exercise study days during the exercise period (60–90 min [-2.1 ± 3.9 vs +1.5 ± 6.2 pmol/l/h; $p=0.507$]) or post-exercise (90–210 min [-162.9 ± 58.5 vs -122.1 ± 40.6 pmol/l/h; $p=0.780$]). However, ΔAUC glucose was significantly lower on the exercise study day versus the non-exercise day both during the exercise (-0.39 ± 0.11 vs -1.30

± 0.16 mmol/l/h; p=0.001) and post-exercise periods (-3.63 ± 0.97 vs -0.12 ± 0.54 mmol/l/h; p=0.001).
**Conclusion:** A 30-minute period of acute intensive exercise (cycling) did not affect the absorption of sc IGlarg injected into the thigh in people with Type 1 diabetes. This lack of exercise effect on insulin glargine absorption is also reflected in the plasma insulin areas, which did not differ significantly between the exercise and non-exercise study days. However, exercise attenuated the glucose response area (ΔAUC) both during and after the exercise period, suggesting that the decrease in glucose levels occurs independently of insulin mobilisation from the basal insulin depot site.
*Supported by: Aventis Pharma.*

# OP 15
# Diabetic neuropathy

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**The nerve axon reflex-vasodilation for the assessment of small nerve fibers' dysfunction in diabetic patients**
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**Background and aims:** Small fiber neuropathy remains a diagnostic challenge since currently available test methods are not objective and have a high variability. Nerve axon reflex-related vasodilation (N-V response) has been recently proposed as an objective method to quantify c-nociceptive fibers'function. The aim of the present study was to validate the N-V response for the assessment of small fiber function compared to the currently employed techniques.
**Materials and methods:** Twenty-six diabetic patients (16M/10F) without peripheral vascular diseases were recruited from our unit. The neuropathy evaluation included the assessment of the Neuropathy Symptom Score (NSS), the Neuropathy Deficit Score (NDS), the Vibration Perception Threshold (VPT), the Heat Detection Threshold (HDT), nerve conduction studies and standard cardiovascular tests. The direct and indirect (N-V response) vasodilation to iontophoresis of 1% acetylcholine chloride solution was assessed at the forearm and both feet by a two-single point Laser Doppler probes. The direct and indirect vasodilation to iontophoresis of 1% sodiumnitroprusside solution was assessed as well.
**Results:** A significant correlation was observed between the N-V response at the foot level and VPT ($r = -0.462$, $p<0.02$), NDS ($r=-0.525$, $p<0.01$), HDT ($r=-0.571$, $p<0.005$), nerve conduction velocity of the tibial and sural nerves ($r=0.594$, $p<0.005$, $r=0.485$, $p<0.005$ respectively), amplitude of the sural nerve ($r=0.634$, $p<0.005$) and the Valsalva test ($r=0.533$, $p<0.02$). No correlation was found between the N-V response at the foot and NSS. No correlations were found between the other vascular responses at both the forearm and feet and any of the nerve function measurements. Diabetic patients without neuropathy had a significantly higher N-V response at the foot compared to those with mild, moderate and severe neuropathy (84.8 ± 58 vs, 30.4 ± 26, 14.9 ± 10, 26.2 ± 15; p=0.01, percentage of increase over baseline blood flow), stratified on the basis of the NDS. Finally, a N-V response <50% showed to be highly sensitive (92%), though less specific (67%), in identifying patients with peripheral neuropathy, diagnosed according to the criteria of the Consensus Conference of San Antonio.
**Conclusion:** We conclude that the N-V response assessment is a reliable method to diagnose small fiber dysfunction. This neuro-inflammatory response is reduced even in the early stages of peripheral neuropathy, supporting the hypothesis that small fiber neuropathy precede large fiber impairment.

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**Predictors of depression in neuropathy: a longitudinal study**
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**Background and aims:** The association between diabetic peripheral neuropathy (DN) and depression was assessed longitudinally in 335 patients (mean age = 63 y; 71% male). Also investigated were the mediating roles of neuropathic symptoms, limitations in activities of daily living (DN-ADL) and DN-related family role disruptions.
**Materials and methods:** Measures were completed at baseline, 9 months, and 18 months. DN was diagnosed by the Neuropathy Disability Score (NDS; mean = 7.4). Depression (baseline mean = 5.0) was measured by the Hospital Anxiety and Depression Scale, HADS_D.
**Results:** Controlling for baseline HADS_D and demographic/disease variables, NDS at baseline predicted 18-month HADS_D (beta= .09, p = .037). The association of NDS to 18-month HADS_D was mediated by baseline unsteadiness, which was significantly associated with 18-month HADS_D (beta=.16; p=.001) as was baseline DN-ADL (beta=.19; p<.001). Increases

in pain, unsteadiness, and DN-ADL from baseline to 9 months each significantly predicted increases in 18-month HADS_D (beta=.10; p=.013; beta=.12; p=.007; and beta=.11; p=.017, respectively). Finally, increases in family role restrictions significantly predicted 18-month HADS_D (beta=.13; p=.003) and mediated the relationships of pain, unsteadiness, and DN-ADL changes with 18-month HADS_D. The full model explained 60% of the variance in 18-month HADS_D with 11% of incremental variance accounted for by factors other than baseline HADS-D.
**Conclusion:** These results suggest that DN is a major risk factor for depression, and that psychosocial factors are important predictors of this association. Unsteadiness is the symptom with the strongest association to depression, and it is linked to depression via decreases in functional capacity and in turn by deterioration in social roles.
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**The reliability and validity of a self-administered version of the neuropathy total symptom score-6 (NTSS-6-SA)**
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**Background and aims:** The Neuropathy Total Symptom Score (NTSS-6) assesses the frequency and intensity of six positive sensory symptoms of diabetic peripheral neuropathy (DPN) through face-to-face interviews between a patient and a clinician. We developed a self-administered version (NTSS-6-SA) that can be completed without clinician involvement.
**Materials and methods:** We fielded an 11-page questionnaire that included the NTSS-6-SA to 4000 randomly selected members of the Kaiser Permanente Northwest diabetes registry. We stratified respondents by NTSS-6-SA results and invited a random subset to receive monofilament and VDT assessments three weeks later. Prior to those assessments, but at the same visit, participants again completed the NTSS-6-SA, followed approximately 20 minutes later by the nurse-administered NTSS-6. We evaluated NTSS-6-SA reliability assessing internal consistency (Cronbach's alpha) and test retest reproducibility using intraclass correlation coefficient (ICC). Criterion validity of the self-administered version was assessed by comparing it against the original clinician-administered version. Clinical responsiveness and convergent validity of the NTSS-6-SA were evaluated against two objective measures of DPN, the Semmes-Weinstein 5.07 monofilament test and the CASE IV Vibratory Detection Threshold (VDT) test.
**Results:** 2388 (59.7%) subjects returned completed questionnaires, 409 appeared for follow-up visits and 394 completed all three administrations of the NTSS-6, the monofilament test and the VDT assessment. Internal consistency (Cronbach's alpha) was 0.91 in each of the three administrations. The 3-week test-retest reproducibility of the NTSS-6-SA was 0.88, both against itself and against the NTSS-6. The criterion validity of the NTSS-6-SA against the original NTSS-6 was 0.98. Clinical responsiveness and convergent validity were demonstrated by correlations of the NTSS-6-SA on three occasions with monofilament (r=0.39, r=0.39, and r=0.38, p<0.001) and vibration detection threshold as expressed by normal deviates (r=0.32, r=0.29 and r=0.28, p<0.001). 284 subjects were 100% sensitive to the monofilament (n=284), which reduced these coefficients. Of these 284 subjects, 40.1% scored 6 or greater on the NTSS-6-SA, indicating frequent moderate to severe symptoms of DPN without a discernable loss of protective sensation measured by the monofilament.
**Conclusion:** The NTSS-6-SA is a highly reliable alternative to the clinician-administered NTSS-6. It also demonstrates excellent reproducibility across time and very high internal consistency. The NTSS-6-SA demonstrates clinical responsiveness in relation to objective measures of DPN – but it also appears sensitive to positive sensory symptoms not identified by monofilament testing. Therefore, the NTSS-6-SA could have broad clinical applicability, helping to raise clinicians' and patients' awareness of the symptoms of DPN and their treatment.

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**Validation of a novel screening device for quantitative assessment of small nerve fibre dysfunction as an early feature of diabetic polyneuropathy**
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**Background and aims:** Small nerve fibre dysfunction which is a frequent feature of diabetic distal symmetric polyneuropathy (DSP) can only be detected by measuring warm and cold thermal perception thresholds (TPT) using time-consuming and expensive equipment. We developed and validated an instrument (NeuroCheck) for quantitative bedside testing of cold TPT based on the wind chill factor, i.e. the effect that wind has on our perception of cold. This handheld microprocessor-operated electronic device comprises a fan adjustable to rotate at 10 different velocities, while a constant distance to the skin (23 cm) is ensured by laser diodes.
**Materials and methods:** The NeuroCheck cold TPT (NC-TPT) was examined on the foot in 160 healthy subjects aged 45.5 ± 19.4 years as well as 60 diabetic patients (Type 2: 55%) without DSP aged 48.7 ± 13.6 years and 128 (Type 2: 65%) with DSP aged 58.0 ± 13.1 years. DSP was diagnosed using modified Dyck's critera by standardized measures including a neurological examination, motor and sensory nerve conduction velocity (M/SNCV), vibration perception threshold (VPT: Vibrameter), and warm and cold TPT (Medoc device: method of limits) in the lower and upper limbs. In addition, a C-64 Hz tuning fork and Tiptherm device (for qualitative cold perception) were employed as bedside tests.
**Results:** In the total diabetic cohort the NC-TPT correlated significantly with all nerve function tests, the highest correlation coefficients being found on the foot vs Medoc warm TPT (r=0.618; p<0.001) and cold TPT (r=0.529; p<0.001). The AUCs of the ROC curves for sensitivity and specificity in the diagnosis of DSP were comparable between the three quantitative measures of small fibre function on the foot: NC-TPT: 0.764, Medoc warm TPT: 0.738, Medoc cold TPT: 0.795, but AUC for Tiptherm was smaller: 0.603. When using 3 SD as a conservative cut-off to define the age-related upper limits of normal for NC-TPT, 34% of the patients with DSP had abnormal NC-TPT but normal Medoc warm TPT, whereas only 5% showed the opposite constellation (p<0.05). Likewise, the corresponding percentages for Medoc cold TPT were 32% and 11% (p<0.05), for Tiptherm 47% and 2% (p<0.05), and for the tuning fork 29% and 10% (p<0.05), while no significant differences were noted when comparing NC-TPT to (12% vs 19%), sural SNCV (11% vs 19%), and malleolar VPT (16% vs 13%).
**Conclusion:** Thermal thresholds determined by the NeuroCheck screening device are highly correlated with those obtained by thermal testing using sophisticated equipment. The NeuroCheck is a useful tool for bedside quantitative assessment of small fibre dysfunction and appears to be more sensitive in detecting diabetic polyneuropathy than both elaborate thermal testing and available bedside tests such as the tuning fork.

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**Spinal cord atrophy in diabetic neuropathy, a comparison with healthy volunteers and hereditary sensory motor neuropathy.**
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**Background and aims:** The pathogenesis of diabetic neuropathy remains poorly understood. Long considered a disease of the peripheral nervous system, we have demonstrated that the cross-sectional area (CSA) of the cervical spine was equally reduced in both subclinical (**Sub-DN**) and established DN (**Est-DN**) subjects compared with diabetic patients with no DN. A comparison of spinal cord CSA between a cohort with diabetic DN and a disease control group of hereditary sensory motor neuropathy (**HSMN**) and healthy volunteers (**HV**) will provide further insights into the significance of spinal cord involvement in DN.
**Materials and methods:** 90 male, type 1 diabetic patients underwent clinical and neurophysiological assessment (NIS(LL)+7) to stage DN severity (30 **No-DN** (Dyck's stage N0); 30 **Sub-DSP** (Dyck's stage N1a), and 30 **Est-DSP** (Dyck's stage N1b/2 ). All subjects underwent MR imaging of their cervical spine using a standard spinal phased-array receive-only RF coil on a system operating at 1.5 T (Eclipse, Philips Medical Systems). T2*- weighted imaging was performed axially from C1-T2 using a gradient echo technique (TE = 17.9 ms, TR = 800 ms; $\alpha = 40°$; slice thickness = 4 mm; in-plane resolution = 0.78 mm x 0.96 mm). Cord CSA was measured at the level of disk space C2-C3 in all subjects. Images were post-processed using a semi-automated computerised technique on a Sun Workstation with the image display program Dispimage. Calculated CSA were then corrected for errors in slice positioning and as this is a cross-sectional study, corrections for shrinkage over time were also made.
**Results:** Anova analysis of means between the groups was significantly different (**No-DSP**, **Sub-DSP**, **Est-DSP** and **HV**; p<0.001). Mean CSA and volume measurements were lower in both **Sub-DSP** and **Est-DSP** groups compared to **No-DSP** or **HV** (p<0.0001). No significant difference was found between the two DN groups (**Sub-DSP** vs **Est-DSP**; p=0.80); and between **No-DSP** and **HV** (p=0.71). Furthermore, 15% of **Sub-DSP** and 16% of **Est-DSP** had cord atrophy (defined as CSA below 2SD of the mean CSA of **HV**). In addition there was an early reduction in cervical cord medio-lateral

diameter compared to antero-posterior diameter (p = 0.025), in **Sub-DSP** groups. The mean CSA of the **HSMN** cohort [n = 3; 71.2 (6.8)] appears similar to the **No-DN** [n = 30; 67.34 (6.9)] and **HV** cohorts [n = 20; 68.31 (8.5)].
**Conclusion:** We have demonstrated early involvement of the central nervous system in DSP, reflected in marked reduction in cervical cord CSA. The early reduction in cord medio-lateral diameter seen in **Sub-DSP** suggests a preferentially early involvement of the spinothalamic (small fibre; pain and temperature) tracts. The comparable cord CSA seen in **HSMN, No-DN** and **HV** suggests that the pathogenesis of DSP is not limited to the peripheral nerves and spinal cord involvement occurs concomitantly. However this finding will need to be confirmed with a larger **HSMN** cohort. Finally this non-invasive, reproducible and rapid test may serve as an early diagnostic test for DSP.
*Supported by: Diabetes UK*

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**Oxidative stress variables in sural nerve biopsies of diabetic patients – lessons from a Phase 2 randomized, double-blind, placebo controlled fidarestat dose-finding study**
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**Background and aims:** Animal studies revealed that 1) oxidative stress plays a key role in the pathogenesis of peripheral diabetic neuropathy (PDN), 2) GSH is one of the best markers of oxidative injury in diabetic nerve, and 3) oxidative stress variables (GSH, antioxidative enzymes) inversely correlate with age, blood glucose, and sorbitol pathway activity, and positively correlate with nerve function. We assessed GSH concentration, and GSH peroxidase and GSH transferase activities in 125 type 1 and type 2 diabetic subjects with mild to moderate PDN participating in a Phase 2 randomized, double-blind, placebo-controlled, dose-finding study of the aldose reductase inhibitor fidarestat (F).
**Materials and methods:** Baseline $HbA_1C$ values varied between 7.0 and 11.0%, and age from 23 to 64 years. Patients were randomized to placebo, 1 mg, 3 mg, 9 mg and 15 mg daily of F groups. Sural nerve glucose, sorbitol and fructose concentrations were measured by GCMS, GSH by spectrofluorometric method, and antioxidative enzyme activities by spectrophotometric methods validated according to the FDA requirements.
**Results:** At the end of 12-wk F treatment, sorbitol levels were 34–50% lower in F-treated groups vs the placebo group ($P<0.001$). Fructose levels were 26%, 49% and 41% lower in the groups treated with 3 mg, 9 mg and 15 mg F, respectively ($P<0.001$). GSH concentrations in the placebo-treated subjects were 3-fold lower, GSH transferase activity 6-fold lower and GSH peroxidase activity 3.5-lower than in the peripheral nerve of streptozotocin-diabetic rats. All three markers showed high variability, abnormal data distribution, and no significant differences amongst the groups. An inverse correlation was found between GSH peroxidase activity and fructose concentration ($p<0.003$), and marginally significant positive correlation between GSH peroxidase activity and composite nerve conduction velocity ($p<0.082$). In contrast to animal studies 1) oxidative stress markers did not correlate with blood glucose; 2) neither GSH nor GSH transferase correlated with age; 3) there was marginally significant positive (but not inverse, like in animals) correlation between age and GSH peroxidase activity ($p<0.074$). Furthermore, marginally significant positive, but not inverse, correlations were found between 1) GSH and sorbitol concentrations; 2) GSH and fructose concentrations ($p<0.08$ for both), and 3) GSH and GSH peroxidase activity ($p<0.075$), and strong inverse correlation was found between GSH peroxidase and GSH transferase activities ($p<0.000$).
**Conclusion:** Findings in animal models are not always applicable for understanding mechanisms of PDN in humans. From three variables, GSH peroxidase activity correlated best with sorbitol pathway activity and nerve function. In contrast, GSH did not show any correlation with nerve function which may be indicative of a limited value of this parameter for assessment of therapeutic efficacy of ARIs and other therapies of PDN in humans. Recent experimental studies demonstrated a clearly manifest effect of F on new markers of oxidative injury such as superoxide and nitrotyrosine production and PARP activity. Longer treatment with doses of F resulting in a more robust sorbitol pathway inhibition and, maybe, assessment of other markers of oxidative injury may be needed to document effects of AR inhibition on oxidative stress in advanced PDN in humans.

# OP 16
# GLP 1: in vitro action

## 91

**Glucagon-like peptide-1 secretion is potently stimulated by glutamine in the GLUTag cell line**
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**Background and aims:** Intestinal neuroendocrine L-cells secrete Glucagon-Like Peptide-1 (GLP-1) and Peptide YY (PYY) in response to food ingestion. As GLP-1 enhances insulin release and PYY increases satiety, these peptides are under investigation as candidates for the treatment of type 2 diabetes and obesity. Agents that enhance secretion from intestinal L-cells could provide an alternative treatment strategy. As glutamine is an important metabolic fuel for the gut, the aim of this study was to investigate the effect of glutamine on the GLP-1-secreting cell line, GLUTag.
**Materials and methods:** GLP-1 release was measured following incubation of GLUTag cells under a range of conditions. Electrical activity and membrane currents were studied by perforated patch whole cell recordings. Intracellular free $Ca^{2+}$ was measured in single cells by fura2 fluorescence. Changes in free cytosolic ATP concentration were measured in cells infected with an adenovirus encoding firefly luciferase by photon counting imaging.
**Results:** Glutamine was a more potent GLP-1 secretagogue than glucose or other amino acids, increasing GLP-1 release $7.1 \pm 0.7$ fold (n=19) at 10 mM, with an estimated $EC_{50}$ between 0.1 and 1 mM. In patch clamp recordings, glutamine induced a $Na^+$-dependent inward current of $3.2 \pm 1.2$ pA per cell (n=9), triggering membrane depolarisation and action potential firing. This was mimicked by asparagine and was abolished when extracellular $Na^+$ was replaced with $NMDG^+$. Glutamine also triggered a $Na^+$-dependent increase in the intracellular $Ca^{2+}$ concentration, from $96 \pm 4$ to $827 \pm 49$ nM (n=87). Consistent with the idea that the glutamine-dependent current and $Ca^{2+}$ transient are associated with the activity of a $Na^+$-dependent transporter, a strong RT-PCR band was obtained for the electrogenic glutamine transporter, ATA-2, with weaker bands for a number of other $Na^+$-dependent glutamine transporters. Whereas asparagine and alanine produced electrophysiological and $Ca^{2+}$ changes that were at least as large as those caused by glutamine, they were less effective GLP-1 secretagogues, suggesting that glutamine also potentiates secretion downstream of the $Ca^{2+}$ signal. A plasma membrane potential independent action of glutamine was confirmed by measuring secretion in the presence of 30 mM KCl + diazoxide. Under these conditions glutamine remained a more potent secretagogue than either glucose or alanine. A possible candidate for potentiation would be a glutamine specific increase in cytosolic ATP concentration, however whilst 10 mM glutamine triggered an increase in ATP, it was less effective than 1 mM glucose. In α-haemolysin-permeabilised cells, the effect of glutamine was not mimicked by its metabolites glutamate, ornithine, citrulline, proline or ammonium. Low concentrations of the glutaminase inhibitor L-DON (20 μM) did not affect the action of glutamine, while higher concentrations (1 mM), which block a range of glutamine utilising enzymes, abolished it.
**Conclusion:** Glutamine acts as both a trigger and potentiator of GLP-1 release, consistent with its role as the major metabolic fuel for the gut. The amplifying action might reflect an effect of glutamine itself, or the product of an alternative metabolic pathway. The results suggest that nutritional agents like glutamine might have beneficial effects in diabetes and obesity.
*Supported by : Diabetes UK and The Wellcome Trust*

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**Protein kinase C ζ activation and translocation are required for fatty acid-induced secretion of GLP-1 in intestinal endocrine L cells**
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**Background and aims:** Glucagon-like peptide-1 (GLP-1) is a peptide secreted from the intestinal endocrine L cells that plays an essential role in the regulation of insulin secretion and contributes to satiety after a meal. Recent studies have demonstrated a strong effect of ingested fats on GLP-1 secretion. Protein kinase C (PKC) isozymes such as PKCζ are involved in free fatty acid (FFA) signalling in many cells. We therefore investigated the influence of FFA on GLP-1 secretion and cell signalling, using a murine L cell line (GLUTag).

**Materials and methods:** RNA was isolated from cell samples and RT-PCR was performed. Cells were treated with oleic acid (OA) in serum-free medium supplemented with 0.5% BSA for 2 hours. GLP-1 (7-36NH2) secretion was determined by radioimmunoassay, and intracellular PKCζ localisation was detected by staining with a PKCζ-specific antiserum. To measure PKCζ activity, the cells were starved for 48 hours in serum-free medium, incubated with or without a PKCζ specific inhibitor (ZI) for 24 hours, and then with or without OA. The cells were collected, homogenized, and PKCζ-specific immunoprecipitation was performed, followed by determination of 32P-transfer to a PKCζ -specific substrate.
**Results:** GLUTag cells were found to contain the FFA receptor (GPR40), the FFA transport protein (FATP1) and several isotypes of PKC, including PKCζ, as determined by RT-PCR. OA (250 μM-1000 μM) increased GLP-1 secretion in a concentration-dependent manner, by up to 108 ± 26% of control ($p<0.05$). All further experiments were therefore performed with 500 μM OA. Immunohistochemistry demonstrated the translocation of PKCζ in the cells to the perinuclear area after stimulation with OA. Pretreatment with ZI for 24 h decreased OA-induced secretion by 50%, as compared to OA alone ($p<0.05$). ZI alone also decreased PKCζ activity in the cytosol as well as in the membrane fraction ($p<0.05$ vs. control). OA alone increased PKCζ activity in the cytosol fraction but decreased activity in the membrane fraction ($p<0.05$ vs. control). When ZI was added with OA, PKCζ activity decreased in both the cytosol and membrane fraction ($p<0.05$).
**Conclusion:** These experiments demonstrate, for the first time, that PKCζ activation and translocation are involved in FFA-mediated GLP-1 secretion by enteroendocrine L cells.
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**Characterisation of voltage gated currents underlying the action potential in the glucagon-like peptide-1 secreting GLUTag cell line**
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**Background and aims:** Glucagon-Like Peptide-1 (GLP-1) and Peptide YY (PYY) are released from intestinal L-cells in response to nutrient ingestion. Whereas GLP-1 stimulates insulin release from pancreatic β-cells, PYY enhances satiety. Developing agents that increase secretion from L-cells might therefore provide a novel strategy to treat diabetes and obesity. To adopt this approach, it is essential that we understand the physiological events underlying L-cell function. Previous studies using the GLP-1 secreting cell line GLUTag have shown that the cells are electrically active, and that the frequency of action potential firing is regulated by nutrients. The aim of this study was to investigate the currents underlying action potentials in GLUTag cells.
**Materials and methods:** GLUTag cells were studied using standard and perforated patch whole-cell patch clamp recording techniques. The intracellular and extracellular solutions were varied to isolate the different currents. Intracellular calcium concentrations were measured using fura2 fluorescence. GLP-1 secretion was measured following a 2 hour incubation of GLUTag cells in test agents.
**Results:** GLUTag cells have a resting membrane potential of approximately -50 mV in the absence of nutrient. Action potentials in GLUTag cells were triggered by a variety of nutrients, including glucose and glutamine. The threshold for action potential triggering was around -30 mV.
The largest component of the voltage gated inward current was attributable to the activity of voltage gated $Na^+$-channels. TTX-sensitive currents developed during depolarisations beyond -40 mV and reached a peak amplitude of -661 ± 113 pA/cell (n=13) at +10 mV. Steady state inactivation of this current was half-maximal at -40 ± 1 mV (n=12). Activation and inactivation was fitted assuming $m^3h$ kinetics according to Hodgkin and Huxley and both activation and inactivation time-constants became progressively faster at more depolarising potentials. However, TTX failed to inhibit glucose triggered GLP-1 secretion.
Voltage-gated calcium currents were of smaller amplitude, with a peak of 72 ± 11 pA/cell at 0 mV when measured in 2.6 mM external $Ca^{2+}$ (n=5). They were activated with a threshold of -40 mV, and did not exhibit voltage-dependent inactivation. $Ba^{2+}$-currents were blocked by 10 mM $Co^{2+}$ or 5 μM nifedipine, indicating that they were attributable to the activity of L-type $Ca^{2+}$-channels. Consistent with a role of calcium entry through L-type $Ca^{2+}$-channels in mediating secretion, glucose-triggered calcium transients and GLP-1 release were inhibited by nifedipine.
More than one type of $K^+$-current was observed. We previously reported the presence of ATP-sensitive $K^+$-channels in GLUTag cells. In addition, we observed TEA-sensitive delayed rectifier $K^+$-currents and TEA-insensitive, but 4-AP-sensitive A-type $K^+$-currents. The latter had a threshold of activation of -40 mV, reached a peak amplitude of 170 ± 50 pA/cell at depolarisations to +20 mV and steady state inactivation was half-maximal at -61 ± 2 mV (n=9).
**Conclusion:** The inward current of the action potentials in GLUTag cells is largely carried by $Na^+$ ions. $Ca^{2+}$ entry through L-type calcium channels, however, is essential for GLP-1 secretion, as in other endocrine cells. Understanding the nature of the currents in GLP-1 secreting cells might facilitate the development of agents to enhance GLP-1 and PYY secretion in vivo.
*Supported by: Diabetes UK and The Wellcome Trust*

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**Effect of GLP-1 on glucose transport and its cellular signalling in rat skeletal muscle**
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**Background and aims:** GLP-1 activates PI3K/PKB, p70s6k and p44/p42 MAPKs, and exerts insulin-like effects in rat and human skeletal muscle, where the peptide increases glycogen synthase *a* activity and glucose metabolism; these actions seem to be mediated by at least the activation of PI3K/PKB, and possibly PKC. In this work, we explored the effect of GLP-1 upon glucose transport (GT) in rat skeletal muscle, and the possible participation of kinases proposed to be implicated in the insulin action.
**Materials and methods:** *Soleus* muscle from Wistar rat was used for each experiment. For GT – $^3$H-2DOG uptake –, paired muscle samples (2 per rat) were incubated for 60 min in KRB-HEPES containing 0.5 M 2-deoxi-D-[1,2-$^3$H]glucose (Sp. Ac. 40 nCi/μmol) and 2 M [$^{14}$C]sorbitol (Sp. Ac. 5 nCi/μmol), and in three different additional conditions: a) in the absence (control) and presence of GLP-1 ($10^{-9}$ and $10^{-8}$ M) or $10^{-9}$ M insulin, b) in the presence of GLP-1 or insulin, and without and with inhibitors – $10^{-6}$ M wortmannin (W), $10^{-7}$ M rapamycin (RAP), $2.5 \times 10^{-5}$ M PD98059 (PD), $10^{-4}$ M H-7 and $5 \times 10^{-8}$ M TNFα – of respective kinases – PI3K, p70s6k, MAPKs, PKC y PP-1 –, and c) without and with each inhibitor. Data was normalized and referred to the control value obtained in the muscle samples incubated without added hormone and inhibitor.
**Results:** GLP-1, at $10^{-8}$ M, stimulated GT (130 ± 6% control, n=9, $p=0.023$) as did $10^{-9}$ M insulin (131 ± 9% control, n=9, $p=0.02$); at $10^{-9}$ M GLP-1, no stimulation could be detected; inhibition of PI3K activity by W reduced ($p<0.02$ or lower), without abolishing, the GLP-1 and insulin increasing effect; inhibition of p70s6k activity by RAP, and that of p44/42 MAPKs by PD, completely blocked the stimulatory effect of both GLP-1 and insulin; H-7 partial but significantly diminished ($p<0.03$ or lower) both GLP-1 and insulin action, like it did inhibition of PP-1 activity by TNFα ($p<0.04$ or lower), although the reduction of the effect was total in the case of insulin.
**Conclusion:** In rat skeletal muscle, GLP-1 stimulated glucose transport with an apparent lower potency than insulin. In this action of GLP-1, an activation of p70s6k and MAPKs was required, while those of PI3K, PP-1, and possibly PKC were only partially needed. The partial participation of PP-1 in the effect of GLP-1 is in contrast to that of insulin, in which, full activation of this protein phosphatase seems to be determinant.

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**Signalling in the GLP-1 and exendins action on glucose transport in myocytes from Type 2 diabetic patients**
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**Background and aims:** GLP-1, like insulin, stimulates glucose transport (GT) and glycogen synthase *a* activity in normal human skeletal muscle, apparently through MAPKs, PI3K/PKB and p70s6k activation. Exendin-4 (Ex-4) and its fragment 9-39 amide (Ex-9), reported to be agonist and antagonist, respectively, of the GLP-1 receptors in several cell systems, both have GLP-1-like effect upon GT and metabolism in normal human muscle cells. In this effect both share with GLP-1 the implication of PI3K/PKB and MAPKs. Here we explored, in myocytes from type-2 diabetic patients, GT and activation of kinases by GLP-1 and both exendins.
**Materials and methods:** Myotubes were established from satellite cells of dissociated *vastus lateralis* from 15 type-2 diabetics patients (13F/2M; age: 78 ± 3 yr; fasting plasma glucose: 182 ± 14 mg/dl; cholesterol: 169 ± 17 mg/dl; triglycerides: 115 ± 23 mg/dl; $HbA_{1c}$>6.5%) and 6 normal subjects (5F/1M; age: 80 ± 1 yr; fasting plasma glucose: 106 ± 5 mg/dl) previous informed consent given, undergoing orthopedic surgery. We measured

PI3K – PIP3 formation –, PKB, p44/42 MAPK and p70s6 k phosphorylation – Western blot – and GT – 2 deoxy-D-[1,2-$^3$H]glucose uptake – after 3 and 5 min, respectively, in the absence (control) and presence of $10^{-10}$-$10^{-9}$ M (Western blot) and at $10^{-8}$ M (for GT) peptide.
**Results:** In diabetic patients, compared to normals, only the control value of PI3K was higher ($p<0.0001$); GLP-1 normaly increased ($p<0.05$ or lower) PI3K (45 ± 6% Δ of control), PKB (42 ± 12), p70s6 k (104 ± 41) and MAPKs activity (p42: 51 ± 14; p44: 79 ± 13); both Ex-4 and Ex-9 induced a higher ($p<0.0001$) increment of p44/42 MAPK (Ex-4, p42: 64 ± 19 and p44: 116 ± 34; Ex-9, p42: 68 ± 26 and p44: 176 ± 43); Ex-4 and insulin normally activated PI3K and Ex-9 failed to modify the activity control value, while no activation of PKB could be detected by either exendin; insulin did not activate p70s6 k and MAPKs. Also compared to normal subjects, GT control value was lower (9.1 ± 0.8 fmol glucose/2 × $10^4$ cells, $p<0.014$ *vs* normal: 15.9 ± 0.8), while the respective stimulating effect of GLP-1 (52 ± 8% Δ of control, $p=0.001$), Ex-4 (49 ± 9, $p=0.001$), Ex-9 (61 ± 14, $p<0.0001$) and insulin (76 ± 9, $p<0.0001$) was maintained.
**Conclusion:** Type-2 diabetics have lower basal glucose uptake despite higher PI3K, and both parameters are equally stimulated by GLP-1, insulin and Ex-4. The maintained effect of either exendin on GT seems to be achieved, opposite to insulin, by a higher activation of the MAP kinases pathway.

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**Effect and signalling of GLP-1 action upon glucose transport in adipocytes from Type 2 diabetic rats**
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**Background and aims:** In human adipocytes GLP-1 exerts a counteracting effect on lipid metabolism being lipogenic and lipolytic depending upon the dose, like it does in normal rat fat cells, where GLP-1 also increases glucose uptake. The GLP-1 structurally related exendin-4 (Ex-4) and its fragment 9-39 amide (Ex-9), both are agonist of the peptide action upon glucose metabolism in 3T3-L1 adipocytes, L6 myoblasts and human myocytes, and also of its stimulating effect on glucose transport (GT) and lipogenesis in rat adipocytes, where the three peptides share with insulin an activation of the same initial kinases. In this work we have studied the effect of GLP-1, Ex-4, Ex-9 and insulin on the activity of cellular kinases, and on GT, in adipocytes from type 2 diabetic rats.
**Materials and methods:** Adipocytes were isolated by enzymatic digestion from the epididimal fat of streptozotocin-induced type 2 diabetic Wistar rats. We measured GT – 2-deoxi-D-[1,2-$^3$H]glucose uptake at 16 nM D-glucose – and the activity of PI3K – $PIP_3$ formation –, PKB, p42/44 MAPKs and p70s6 k – Western blot – at 3.3 mM D-glucose, in cells after 3 min incubation in the absence (control) and presence of $10^{-13}$-$10^{-8}$ M GLP-1, Ex-4, Ex-9 or insulin.
**Results:** In type 2 diabetic cells, compared to previously observed in normals, the control PI3K activity was higher (195 ± 9% normal, n=5 rats, $p<0.001$), and so it was the increment induced by $10^{-12}$ M GLP-1, while the respective effect of $10^{-9}$ M GLP-1, Ex-4, Ex-9 or insulin (all peptides, $p\leq0.047$) was of the same magnitude; PKB control value was similar, and in this case not only an increment ($p\leq0.036$) in its phosphorylation by GLP-1, Ex-4 and Ex-9 was measured – which could not be detected in normal rats –, but the magnitude of the stimulation by insulin was even higher ($p=0.026$ *vs* normal); no differences in p70s6 k and p42/44 MAPKs activities, control or stimulated by GLP-1 or Ex-9, were observed, whereas a higher increment on p70s6 k by insulin ($p=0.039$ *vs* normal) and no effect by Ex-4, was detected; moreover, insulin did not modify MAPKs activity. Also as compared to normal rats, no difference in the GT control value was measured (34.5 ± 1.4 fmol/$10^5$ cells, n=12); either peptide stimulated GT in a dose-related manner, GLP-1 ($10^{-12}$ M: 112 ± 4% of control; $10^{-11}$ M: 125 ± 5; $10^{-10}$ M: 132 ± 4; $10^{-9}$ M: 143 ± 5; $10^{-8}$ M: 139 ± 4, n=4, $p\leq0.045$ at all concentrations) being not only more potent than Ex-4 (110 ± 4; 114 ± 5; 117 ± 4, $p=0.042$; 125 ± 4, $p=0.001$; 124 ± 3, $p=0.001$, respectively, n=4), Ex-9 (103 ± 7; 125 ± 6, $p=0.013$; 146 ± 9, $p=0.001$; 137 ± 8, $p=0.001$; 139 ± 7, $p=0.001$, respectively, n=4) or insulin (97 ± 4; 103 ± 3; 119 ± 6, $p=0.003$; 128 ± 6, $p=0.001$; 132 ± 5, $p=0.001$, respectively, n=5), but also more efficient than in normal rats (at $10^{-9}$ and $10^{-8}$ M GLP-1: $p\leq0.025$ *vs* normal), this last opposite to insulin and Ex-4, whose respective increasing effects were less pronounced (at $10^{-9}$ and $10^{-8}$ M: $p\leq0.002$ *vs* normal); no differences with normal rats were detected in the Ex-9 action at any concentration tested.
**Conclusion:** In adipocytes from type 2 diabetic rats, GLP-1 exerts a more potent and efficient effect upon GT than insulin and Ex-4, by which a lower activation than that induced in normal rats is detected; this greater sensitivity of the diabetic fat cell to GLP-1 could be due to an increased PI3K activity, the lower effectiveness of insulin being, perhaps, motivated by a defect in the MAPKs upstream mechanism.

# OP 17
# Beta cell damage

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**Beta cell selective knock out of the NFκB subunit p65 protects mouse islet cells from damage by inflammatory cytokines**
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**Background and aims:** Immune-mediated destruction of autologous pancreatic beta cells represents the central pathogenetic process leading to the manifestation of human type 1 diabetes. As shown in model systems of the disease, major triggers of beta cell damaging pathways are nitric oxide (NO) and cytokines such as tumor necrosis factor α (TNFα), interleukin-1β (IL-1β) and interferon γ (IFNγ) which are released during the beta cell-directed immune reaction. The aim of our study was the elucidation of the potential role of the nuclear transcription factor NFκB in beta cell death induced by inflammatory cytokines.
**Materials and methods:** By the use of a complex Cre-lox targeting strategy we generated a mouse line carrying a beta cell selective knock out of the regulatory subunit p65 of the nuclear transcription factor NFκB. Isolated islet cells from mice with functionally active NFκB ($p65^+$) or with beta cell selective p65 defect ($p65^-$) were exposed to the NO donor DETA-NO, to the beta cell toxin streptozotocin (SZ), or to a mixture of the inflammatory cytokines TNFα, IL-1β and IFNγ.
**Results:** As determined by trypan blue exclusion, p65 deficiency did not affect islet cell susceptibility to DETA-NO or SZ. $P65^+$ cells exposed to 0.2 mM DETA-NO or 1.5 mM SZ (24 h) were lysed to 18.7 ± 5.3% and 55.0 ± 3.1%, respectively. Similar levels of cell death were observed in $p65^-$ cells after DETA/NO- (30.2 ± 6.4%) or SZ-exposure (57.6 ± 7.0%). In contrast, p65 deficiency significantly protected from cytokine-mediated damage. Exposure to a mixture of 5 U/ml TNFα, 0.5 U/ml IL-1β and 1 U/ml IFNγ (6 d) caused the death of 33.9 ± 3.8% $p65^+$ cells but of only 8.5 ± 1.6% $p65^-$ cells ($p<0.0001$). Protection of $p65^-$ cells was evident even at 10 times higher cytokine concentrations (dead $p65^+$ cells: 70.9 ± 7.6%; dead $p65^-$ cells: 9.3 ± 0.4% ($p<0.0001$)). Comparable nitrite concentrations in the cultures of $p65^+$ (3.3 ± 0.1 μM nitrite) and $p65^-$ cells (3.4 ± 0.1 μM nitrite) demonstrate that cytokine-induced NO production is independent of p65 expression.
**Conclusion:** Our results obtained with islet cells from animals with beta cell selective NFκB defect indicate that NFκB does not affect the toxicity of NO released from exogenously applied mediators but controls an NO-sensitive regulatory step involved in cytokine-mediated beta cell damage.

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**Suppressor of cytokine signalling (SOCS)-3 inhibits cytokine induced NFκB activation, apoptosis and nitrogen oxide (NO) production in pancreatic beta-cells**
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**Background and aims:** The pro-inflammatory cytokines IL-1β and IFNγ are believed to be mediators of immune-mediated beta-cell destruction in type 1 diabetes. Inhibition of the primary pro-apoptotic signalling pathways in beta-cells, MAPK/JNK and NF-κB, protects the beta-cells against cytokine induced apoptosis, in part due to inhibition of inducible Nitrogen Oxide Synthase (iNOS) expression and resulting NO generation. Induction of recombinant Suppressor of Cytokine Signaling (SOCS)-3 inhibits IL-1β as well as IFNγ induced apoptosis in INS-1 beta-cells. Whereas SOCS-3 inhibition of IFNγ signalling is well described, the mechanisms responsible for IL-1β inhibition remain unknown. The aims of this study were therefore: a) to investigate by MicroArray analysis the effect of induced SOCS-3 expression on the general IL-1β induced transcription profile in a beta-cell line, and b) to specifically addressed the ability of SOCS-3 to inhibit IL-1β activated signalling through MAPK (ERK, p38, and JNK) and NF-κB activation, as well as NO production and apoptosis in primary rat beta-cells.

**Material and methods:** For the MicroArray analysis we used a beta-cell line with doxycycline inducible SOCS-3 expression. The cells were exposed to IL-1 (150 pg/ml) for 6 hrs with or without addition of doxycycline. Gene expression was determined (n=2) for each condition by hybridising 10 μg labelled total RNA to Affymetrix GeneChip U34A Array, each containing gene probe pairs for >7000 known genes and >1000 ESTs. Differential expression was determined by comparison of arrays for each condition with the control arrays. For the signalling studies, dispersed adenovirus transduced primary rat islet cultures were used (n=4-6).
**Results:** We found that IL-1 up-regulated 23 known genes, 15 of which were significantly inhibited by SOCS-3. Interestingly, of these 15 genes, 9 have previously been reported to be NF-κB dependent. To study the effect of SOCS-3 in primary beta-cells, SOCS-3 was expressed in dispersed rat islet cultures following adenoviral transduction. IκB degradation and MAPK activation were analysed by Western Blotting of lysates from cells exposed to IL-1β (150 pg/ml) for 15 min. In support of the MicroArray analysis, suggesting SOCS-3 inhibition of IL-1β induced NFκB signalling, IL-1β induced IκB degradation and MAPK activation was found to be inhibited by SOCS-3. In addition, IL-1β (250 pg/ml, 24 hrs) or IL-1β + IFNγ (250 pg/ml and 10 ng/ml, 24 hrs) induced apoptosis and NO production was suppressed by approximately 50% in cultures transduced with SOCS-3 encoding adenovirus, compared to cultures either non-tranduced or transduced with a luciferase expressing adenovirus.
**Conclusion:** Using MicroArray technology we show that expression of SOCS-3 suppresses IL-1β induced NF-κB dependent gene transcription in a beta-cell line, and inhibits IκB degradation and MAPK activation in primary rat beta-cells. These findings elucidate the signaling pathways involved in the mechanism by which SOCS-3 reduces IL-1β induced apoptosis and NO generation in beta-cells, which may be useful in future intervention strategies.
*Supported by : a research grant from the Juvenile Diabetes Research Foundation*

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**Cytokines downregulate the sarcoplasmic-endoplasmic-reticulum pump $Ca^{2+}$-ATPase (SERCA)2b and deplete endoplasmic reticulum (ER)-$Ca^{2+}$ leading to ER-stress in pancreatic beta-cells**
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**Background and aims:** Cytokines and free radicals are early mediators of beta-cell death in type 1 diabetes mellitus. Under *in vitro* conditions, the cytokines IL-1β+IFN-γ induce nitric oxide (NO) production and apoptosis in rodent and human pancreatic beta-cells. We have previously shown, by microarray analysis of primary beta-cells, that IL-1β+IFN-γ decrease expression of the mRNA encoding for the ER $Ca^{2+}$ pump SERCA2b, while inducing expression of the ER stress-related and pro-apoptotic gene CHOP. The aim of this study was to evaluate whether cytokine-induced downregulation of SERCA2b leads to a decrease in ER $Ca^{2+}$ and activation of the major ER stress pathways in beta-cells.
**Material and methods:** FACS purified rat beta-cells and INS-1E cells were exposed to IL-1β (10–50U/ml), IFN-γ (100U/ml) and/or the iNOS inhibitor $N^G$-methyl-L-arginine (LMA; 1 mM). The SERCA inhibitor thapsigargin (1-2 μM) was used as a positive control for ER stress. Cell viability was evaluated by HO 342 and propidium iodide. Cytosolic and ER $Ca^{2+}$ concentrations were determined using FURA-2 and furaptra, respectively. Expression of genes/proteins related to ER stress were determined by real time RT-PCR and Western blot, while activation of ATF6 was evaluated using a 5xATF6-luciferase reporter construct.
**Results:** Exposure of FACS-purified rat beta-cells to IL-1β+IFN-γ for 3 and 6 days induced apoptosis in 52% and 66% of the cells respectively, as compared to 8% and 10% in control beta-cells ($p<0.001$; n=4). L-MA prevented IL-1β+IFN-γ-induced apoptosis (13% and 18% after 3 and 6 days; $p<0.001$ vs. IL-1β+IFN-γ; n=4). Thapsigargin induced apoptosis in primary beta-cells already after 24 h of exposure (31% apoptotic cells after thapsigargin (1 μM) vs 8 ± 1% in control; $p<0.01$, n=3). A 6 and 24 h exposure to IL-1β+IFN-γ decreased SERCA2b mRNA expression by 40% and 55% respectively ($p<0.005$ vs. control), and severely depleted ER $Ca^{2+}$ ($p<0.05$ vs control). Both effects were prevented by L-MA. ER $Ca^{2+}$ depletion was paralleled by a NO-dependent induction of CHOP mRNA (5 fold induction by IL-1β+IFN-γ as compared to control; $p<0.01$; n=6) and protein and activation of two major ER-stress pathways, namely IRE-1α (as evaluated by xbp-1 splicing) and PERK/ATF4. Cytokine-induced ER stress response in beta-cells was, however atypical, lacking activation of the ATF6 and JNK pathways. In contrast, the ER stress-inducing agent thapsigargin induced these four ER stress pathways in parallel.
**Conclusions:** Our results suggest that IL-1β+IFN-γ induce a decrease in SERCA2b expression, depletion of ER $Ca^{2+}$ and activation of the ER stress pathway in pancreatic beta-cells. These effects of cytokines are mostly mediated via NO production.
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## 100

**Glucotoxicity in human pancreatic islets is mediated by oxidative stress: evidence for limited defense capacity**
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**Background and aims:** Chronic exposure to high glucose can lead to irreversible damage of beta-cell accounting for increased apoptosis and impaired insulin secretion. Multiple mechanisms concur in generating the damage, but all of them appear to act via oxidative stress, as indicated by studies performed with murine islets. Whether a similar susceptibility exists for human islets is less evident. Therefore, we have determined the effect of 24-hr high glucose incubation of human pancreatic islet on insulin secretory function as well as mRNA expression of several enzymes involved in the generation and scavenging of reactive oxygen species (ROS).
**Materials and methods:** Human islets, prepared by collagenase digestion and density gradient purification from 6 pancreases of multiorgan donors (age 68 ± 6 yrs, sex 3F/3M, BMI 24.2 ± 2.0 $Kg/m^2$), were incubated for 24-hrs in the presence of 5.5 mM (Ctrl) or 22.2 mM (HG) glucose, with or without 0,3 nmol/l glutathione (GSH). At the end of incubation period, insulin secretion was measured by static incubation (expressed as stimulation index, i.e. the ratio of stimulated over basal release), and mRNA expression of insulin, SOD2, SOD3, Catalase, Glutathione peroxidase, HO-1, and subunits p22-phox and gp91-phox of cytochrome $b_{558}$ of NADPH-oxidase by quantitative Real-Time RT-PCR. Nitrotyrosine levels were determined by ELISA.
**Results:** As compared to Ctrl incubation, exposure to HG was associated with impaired insulin release (SI: 1.1 ± 0.3 vs. 2.6 ± 0.3, $p<0.05$), and reduced insulin mRNA expression (-80% respect to Ctrl, $p<0.005$). GSH prevented changes in insulin release (SI: 2.2 ± 0.4) and mRNA expression (+20%; both $p<0.05$ vs. HG). Exposure to HG was associated with oxidative stress as indicated by a significant accumulation of nitrotyrosine (Ctrl: 7.0 ± 0.4, HG=14.8 ± 1.5 nmol/l; $p<0.05$). GSH restrained such increase (9.0 ± 0.6 nmol/l, $p<0.05$ vs. HG). As for insulin, mRNA expression of SOD2, SOD3, Catalase, GSH-Px, and HO-1 were all increased after HG (+110, +180, +300, +120 and + 270% of Ctrl; all $p<0.05$ or less), indicating a full activation of the ROS scavenging system. These changes were much less marked in the presence of GSH (-20, +10, +40, -20 and -30%, respectively; all $p<0.05$ vs. HG). NADPH-oxidase is a potent source of ROS, and mRNA expression of p22-phox and gp91-phox subunits were markedly increased after HG (+90 and +80%, with respect to Ctrl; $p<0.05$ vs. Ctrl). This increase was completely prevented by GSH (both +30%; $p<0.05$ vs. HG).
**Conclusion:** In conclusion, in human islets 24-hr exposure to high glucose causes oxidative stress in spite of prompt transcription of several enzymes involved in the ROS scavenging process, suggesting a limited capability of the system to cope with ROS insult. These observations along with the protective effect induced by antioxidant molecules (GSH) support the hypothesis that oxidative stress is a main mechanism for glucose toxicity in human pancreatic islets.

## 101

**Dominant-negative suppression of SREBP-1c action prevents glucolipotoxicity in INS-1 cells**
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**Background and aims:** The reduction in insulin secretory capacity and β-cell mass observed in Type 2 diabetes is thought to be caused, at least in part, by glucolipotoxicity secondary to hyperglycemia and hyperlipidemia. Elevated expression of sterol regulatory element binding protein-1 c (SREBP-1 c) has been demonstrated in islets and liver of diabetic animals. Lipid accumulation, impaired glucose-stimulated insulin secretion, defective β-cell gene expression (insulin, Pdx-1, glucokinase, and Glut-2), disorganized mitochondrial ultrastructure, and "lipoapoptosis" have been

reported in the β-cells of diabetic animals. Moreover, Leptin, metformin, adiponectin, GLP-1 and PPARγ agonists all prevent overexpression and activation of SREBP-1 c. Thus there is a good correlation between suppression of SREBP-1 c function and antidiabetic effects of these agents. In a cellular model system, we have recently implicated the transcription factor SREBP-1 c in β-cell glucolipotoxicity. We propose now to substantiate this hypothesis by preventing β-cell dysfunction through a dominant-negative suppression of SREBP-1 c action in the INS-1 cell.
**Materials and methods:** We have employed the Tet-On system to establish an INS-1 stable cell line, called DN-SREBP-1 c*23, permitting suppression of SREBP-1 c function through induction of dominant-negative mutant of SREBP1 c (DN-SREBP-1 c). DN-SREBP-1 c contains intact dimerization domain but lacks DNA-binding domain. It thus exerts its dominant negative function by forming non-functional dimmers with endogenous active form of SREBP-1 c.
**Results:** High glucose (30 mM) treatment of INS-1E cells for 48 h caused impaired glucose- stimulated insulin secretion, lipid accumulation and apoptosis. Quantitative Northern blotting demonstrated that this glucotoxicity led to decreased expression of insulin, Glut2, glucokinase, Pdx-1, glucagon-like peptide-1 receptor (GLP-1R), carnitine palmitoyl transferase-1 (CPT-1), and acylCoA oxidase (ACO), as well as elevated expression of lipogenic enzymes and pro-apoptotic genes. These observations are strikingly similar to the consequences of overexpression of the nuclear active form of SREBP-1 c in INS-1 cells. In fact, high glucose was shown to promote the processing of SREBP-1 c from the precusor to its active form. We found that doxycycline-mediated induction of DN-SREBP-1 c in INS-1 cells prevented the inhibitory effects of high glucose on expression of Glut2, glucokinase, Pdx-1, GLP-1R, CPT-1 and ACO. In addition, dominant-negative suppression of SREBP-1 c action also reversed the induction by high glucose of transcripts for lipogenic enzymes: fatty acid synthase and HMGCoA reductase. In contrast, induction of DN-SREBP-1 c did not alter the increased expression of L-pyruvate kinase and aldolase B under the same conditions. Moreover, dominant-negative suppression of SREBP-1 c action in INS-1 cells markedly prevented lipid accumulation, apoptosis and the insulin secretory defect induced by 48 h high glucose treatment.
**Conclusion:** These results suggest that SREBP-1 c is instrumental in the development of β-cell glucolipotoxicity. Prevention of SREBP-1 c action should be useful for therapy aimed at protection of β-cell function in Type 2 diabetes.

## 102

**Anti-apoptotic effect of extracellular matrix on rat primary beta cells involves β1 integrins and signalling via PKB, ERK and FAK**

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**Background and aims:** Rat primary beta cells cultured on 804G matrix (secreted by rat bladder carcinoma) are protected from apoptosis compared to cells plated on poly-L-lysine (pLL). One major component of this matrix is laminin-5. This has led us to investigate the possible role of the β1 integrins, which are cell receptors of laminin-5, in the anti-apoptotic effect of laminin-5/804G matrix.
**Materials and methods:** Rat primary beta cells sorted by FACS were cultured in petri dishes coated with pLL, 804G matrix or laminin-5. The percent of apoptotic cells was measured by TUNEL assay after short-term (4 h) or long-term (48 h) culture. The involvement of the β1 integrins was assessed by pre-incubating the cells in suspension with the blocking anti-β1 antibody Ha2/5 or with control IgM. The activation of intracellular signalling pathways was checked by western blotting using phospho-specific antibodies for PKB, GSK3β, ERK and FAK. The implication of the PI3-kinase and MAPK pathways was confirmed by means of specific inhibitors: LY294002 and PD98059 respectively. Data are means ± SEM, with level of significance assessed by student's t-test.
**Results:** Rat primary beta cells cultured on laminin-5 showed the same beneficial anti-apoptotic effect as cells plated on matrix (1.5% ± 0.6 and 0.5% ± 0.3 TUNEL positive cells, $p = 0.19$) compared to pLL (11% ± 0.8) after 48 h. This result implied a possible prominent role of the β1 integrins. Indeed, pre-incubation with the blocking anti-β1 antibody Ha2/5 induced a reduction of cell spreading on matrix and an increased apoptosis vs control IgM after 4 h (1.9% ± 0.5 vs 4.9% ± 0.6, $p<0.05$). Neither IgM had any effect on apoptosis for cells plated on pLL. Plating cells on matrix induced phosphorylation of several kinases. After 30 min, PKB was highly phosphorylated (on Ser473) inducing the inhibition (by phosphorylation on Ser9) of its substrate GSK3β. The phosphorylation of ERK1/2 (on Thr202/Tyr204) and FAK (on Tyr397) was also increased in cells cultured on matrix compared to pLL. The treatment of cells with 50 μM of either LY294002 or PD98059 showed nearly complete inhibition of the phosphorylation of PKB or ERK1/2 respectively, as determined by western blot. Such inhibition correlated with a significant ($p = 0.04$) increased number of apoptotic cells on matrix (2.4% ± 0.3 for LY294002 and 2.6% ± 0.2 for PD98059 vs 1.3% ± 0.2 for control cells) after 4 h. The effect of PD98059 was yet more evident after 48 h (13.4% ± 3.2), whereas LY294002 was no longer inhibitory (1.3% ± 0.5 vs 0.6% ± 0.2). Neither inhibitor affected apoptosis of cells plated on pLL.
**Conclusion:** The protective effect of 804G matrix on apoptosis is induced by the interaction between laminin-5 and the β1 integrins and mediated, at least in part, by the PI3-kinase, MAPK and FAK pathways.
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# OP 18

# Epidemiology of Type 2 diabetes

## 103

**Smoking is associated with an increased risk of Type 2 diabetes but a decreased risk of autoimmune diabetes: an 11-year follow-up of incidence of diabetes in the Nord-Trøndelag Study**

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**Background and aims:** Epidemiological studies indicate that smoking is a risk factor for type 2 diabetes. Whether smoking affects the risk of autoimmune diabetes has not been investigated. The design of the large prospective Nord-Trøndelag health survey offered a possibility to test this notion.
**Materials and methods:** The Nord-Trøndelag health survey is a large, population-based, prospective study where incident cases of diabetes were classified according to clinical history and presence or absence of glutamic acid decarboxylase antibodies (anti-GAD). In this cohort, we tested for an association between smoking on one hand and the cumulative incidence (risk) of type 2 diabetes and autoimmune diabetes respectively on the other during an 11-year follow-up. Data were available for a cohort of 38,805 men and women who were free of diabetes at baseline and in whom baseline information on smoking, age and body mass index (BMI) and leisure physical activity was also available.
**Results:** Confirming previous reports heavy smoking (>=20 cigarettes per day) carried an increased relative risk (RR) of type 2 diabetes (n=738, RR=1.64, 95% confidence interval (CI)= 1.12-2.39) after adjustment for age, sex and body mass index. In contrast, smoking was associated with a reduced risk of latent autoimmune diabetes in adults (LADA) as well as of traditional type 1 diabetes (LADA n= 81, RR=0.25, 95% CI=0.11-0.60) and type 1 diabetes, n=18, RR=0.17, 95% CI=0.04-0.73). Additional adjustment for physical activity and alcohol consumption did not change these findings.
**Conclusion:** The results indicate that nicotine influences effects of autoimmunity in diabetes. The findings could help elucidate the process of autoimmune beta cell destruction in diabetes.

## 104

**Associations between diet and insulin resistance - The Inter99 study**

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**Background and aims:** The increasing prevalence of Type 2 diabetes is partly explained by increased obesity and physical inactivity but controversy exists about the influence of habitual diet. Insulin resistance is an early marker in the development of Type 2 diabetes and may be influenced by habitual diet. The aim was therefore to identify macronutrients associated with the probability of having insulin resistance.
**Materials and methods:** Baseline data from a large, danish, population-based survey (The Inter99 study) were analyzed (N=6784, aged 30–60 years). The dietary intake was estimated by a self-administered food frequency questionnaire and the homeostasis model assessment was used to calculate the index of insulin resistance. The analyses were based on 6201 subjects where both food frequency questionnaire and index of insulin resistance were available.
**Results:** The associations between macronutrients and insulin resistance were analyzed by multiple linear regression using index of insulin resistance as continuous dependent variable. Fat, carbohydrate, protein and alcohol entered the model as continuous variables in energy percentages with adjustment for age, sex, smoking, physical activity, BMI and waist. The preliminary results show that substitution of alcohol with protein and substitution of alcohol or carbohydrate with fat were associated with increased insulin resistance.

Effects of substituting one macronutrient with another (energy %) on insulin resistance

| Dietary variables | Regression coefficient | 95% CI | P-value |
|---|---|---|---|
| 3 E% protein substituting 3 E% alcohol | 0.037 | (0.012; 0.061) | < 0.01 |
| 3 E% protein substituting 3 E% carbohydrate | 0.024 | (0.000; 0.048) | NS |
| 3 E% protein substituting 3 E% fat | 0.012 | (–0.009; 0.034) | NS |
| 3 E% fat substituting 3 E% alcohol | 0.024 | (0.010; 0.038) | < 0.001 |
| 3 E% fat substituting 3 E% carbohydrate | 0.012 | (0.004; 0.019) | < 0.01 |
| 3 E% carbohydrate substituting 3 E% alcohol | 0.013 | (–0.002; 0.028) | NS |

**Conclusions:** Replacement of alcohol with protein had the most detrimental effect on insulin resistance in the Inter99 population, but substitution of alcohol or carbohydrate with fat were also of importance.

## 105

**Microalbuminuria, diabetes and coronary heart disease risk in an ethnically diverse UK population**

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**Background and aims:** Type 2 diabetes is more prevalent in South Asians and African Caribbeans than in Europeans. Previous reports of risks of microvascular complications are few and conflicting. We studied ethnic differences in a) albumin excretion rates (AER) in men and women with diabetes, impaired glucose tolerance (IGT), impaired fasting glucose (IFG) and normal glucose regulation and b) the relationship between microalbuminuria and prevalent coronary heart disease (CHD) in men.
**Materials and methods:** A combined analysis of two population based cross-sectional studies performed to identical protocols, conducted between 1988 and 1991, of 1,500 Europeans (70% male), 970 South Asians (79% male) and 565 African Caribbeans (51% male) resident in London and aged 40–69 years. Fasting blood, overnight urine collection and clinical measurements were performed. Prevalent CHD was defined by clinical history or major ECG changes.
**Results:** In those with diabetes or IGT or IFG, age and gender adjusted AERs (geometric means, microgm/min) were higher in African Caribbeans (6.9, 95% CI: 6.1, 7.9) than in South Asians (5.0, 95% CI: 4.5, 5.5) and Europeans (5.7, 95% CI: 5.1, 6.4). These ethnic differences persisted in normoglycaemic individuals; geometric means: 5.8 (95% CI: 5.3, 6.3), 3.7 (95% CI: 3.5, 3.9) and 3.9 (95% CI: 3.8, 4.1) in African Caribbeans, South Asians and Europeans respectively. Mean arterial blood pressure and glucose regulation category were the most significant associates of AER in all ethnic groups, but neither these nor any other measured risk factor, could account for the higher AER in African Caribbeans.
Age adjusted prevalences of microalbuminuria (AER>20 microgm/min) were similar in men in all ethnic groups (6–7%). Prevalences of CHD in men were 9% in Europeans, 10% in South Asians and 5% in African Caribbeans. After adjustment for age, glucose regulation category, blood pressure and smoking, presence of microalbuminuria was significantly associated with prevalent CHD in South Asian men (odds ratio (OR): 2.2, p=0.03), but not in African Caribbean men (OR: 0.6, p=0.7), or in European men (OR: 1.2, p=0.6).
**Conclusion:** African-Caribbeans had the highest AERs, not accounted for by higher blood pressure or glucose tolerance category, while South Asians had lower AERs despite adverse risk factor profiles. In South Asian men microalbuminuria was more strongly associated with CHD than in European and African Caribbean men. The unexplained ethnic differences warrant further investigation in prospective studies.

## 106

**Insulin resistance, proinsulin and coronary heart disease: a population-based, follow-up study in 70-year old men using the euglycemic insulin clamp**

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**Background and aims:** No previous longitudinal study has investigated the association between insulin sensitivity (M/I), measured by the euglycemic insulin clamp (EIC) and coronary heart disease (CHD). Some, but not all studies have reported a relationship between plasma insulin and CHD. Recent studies report a relationship between proinsulin and CHD. Conventional insulin assays measure immunoreactive insulin (IRI) including proinsulin-like molecules (PLMs). The aim was to determine the longitudinal relationships between M/I, intact proinsulin, split proinsulin, specific insulin, IRI and subsequent CHD.
**Materials and methods:** Population-based cohort study conducted from August 1991 to May 1995 among 918 men in Uppsala, Sweden, aged 70 years at baseline with a follow-up of up to 10 years using registry data obtained from the National Board of Health and Welfare in Sweden. At baseline, insulin sensitivity, using EIC, was determined. Fasting PLMs and specific insulin concentrations were analysed blinded for outcome, using specific two-site immunometric assays.
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 (International Classification of Diseases [9th revision] codes 410 to 414). Associations were analyzed using Cox's proportional hazards regression, presented as hazard ratios (HRs) with their 95% confidence intervals (CIs) for a one SD increase in a predictor variable.
**Results:** In multivariate analysis, M/I (HR, 0.65, CI, 0.48–0.88), total cholesterol (HR, 1.21, CI, 1.04–1.43), smoking (HR, 1.60, CI, 1.10–2.33) and systolic blood pressure (HR, 1.10, CI, 1.01–1.19) predicted CHD. Intact proinsulin (HR, 1.21, CI, 1.02-1.64) was independent of conventional risk factors whereas specific insulin was not (HR, 1.12, CI, 0.94-1.35).
**Conclusion:** Insulin resistance, i.e. low insulin sensitivity, predicts subsequent CHD. Proinsulin was a good surrogate marker for insulin resistance in predicting CHD.

## 107

**Evaluating SCORE and Framingham fatal coronary heart disease risk equations using UKPDS data**

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**Background and aims:** Accurate coronary heart disease (CHD) risk estimation in people with Type 2 diabetes (T2DM) is needed to help ensure appropriate management. The joint European cardiology societies commissioned the Systematic Coronary Risk Evaluation (SCORE) calculator as an alternative to the Framingham risk equations in Europe. SCORE estimates fatal CHD risk in diabetes by multiplying its risk prediction for the general population by two for men and four for women. We used UK Prospective Diabetes Study (UKPDS) data to determine the accuracy with which SCORE and Framingham estimate fatal and non-fatal CHD risk in diabetes.
**Materials and methods:** 3,898 of 5,102 UKPDS patients with newly presenting type 2 diabetes had sufficient data available for these analyses. Their mean (standard deviation) age, systolic blood pressure, total cholesterol and HDL cholesterol were 52.7 (8.6) yrs, 135 (19)mmHg, 5.36 (1.1) mmol/L and 1.07 (0.24) mmol/L, respectively. SCORE and Framingham equations were applied to baseline risk factors, adjusting for length of follow-up, to estimate the expected number of fatal CHD events in the cohort. The Framingham equations were used also to estimate the expected number of patients with fatal and non-fatal CHD events.
**Results:** During mean 9.8 years follow-up there were 306 fatal CHD events (7.9%, 95% confidence interval 7.0% to 8.7%) and 717 patients had fatal or non-fatal CHD events (18.4%, 95% confidence interval 17.2% to 19.6%). In comparison, SCORE estimated 227 (5.8%) and Framingham estimated 160 (4.1%) fatal CHD events. The Framingham estimate for patients with fatal and non-fatal CHD events was 623 (16.0%.).
**Conclusion:** SCORE and Framingham underestimate the rate of fatal CHD by around a quarter and a half respectively in this well-characterized clinical trial population followed from diagnosis of type 2 diabetes and with complete ascertainment of outcomes. Framingham estimates for fatal and non-fatal CHD events, however, are closer to those observed. Using non diabetes-specific risk calculators, such as SCORE and Framingham, may underestimate the true CHD risk with the result that less intensive diabetic management may be instituted than evidence based guidelines would recommend.
*Supported by: the Healthcare Foundation*

## 108

**Stepwise opportunistic screening for Type 2 diabetes is more effective than mail-distributed population based stepwise screening. ADDITION Denmark**

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**Background:** We have previously shown that population based stepwise screening for unknown T2DM based on mailed invitations is ineffective, primarily because of low response- and attendance rates. Opportunistic screening might be a way to increase response and attendance rates to a screening programme aimed at T2DM. The aim was to compare response- and attendance rates and the yield of opportunistic screening with population based screening using the same screening tools and algorithms.
**Methods:** A stepwise screening algorithm was used in both approaches. The first step was a self-administered risk-chart. The second step consisted of RBG and HbA1 c. The resulting high-risk individuals underwent diagnostic procedures (FBG or OGTT). Patients were classified according to the 1999 WHO classification, based on capillary whole blood glucose. 8 practitioners contributed with 4399 patients aged 40–69 years in opportunistic screening. A poster in the waiting area described the screening programme and a risk chart was handed over to the patients attending practice. The risk chart was filled-in in the waiting area and patients with a risk-score ≥ 5 points were offered the screening tests concomitantly with the subsequent consultation. The results of the opportunistic screening were compared to the outcome of population based screening (where the risk-chart was mail-distributed and high-risk individuals had to make a screening appointment: 60 GP's and 21632 patients participated).
**Results:**

| | Opportunistic | | Mail-Dist-Pop. Based | | |
|---|---|---|---|---|---|
| | N | % DM | N | % DM | p-value |
| Population | 4399 | 0.7 | 21632 | 0.6 | 0.5 |
| Attended practice | 3335 | 0.9 | – | – | – |
| Received risk-chart | 1031 | 2.8 | 21632 | 0.6 | < 0.001 |
| responded | 1029 | 2.8 | 10680 | 1.2 | < 0.001 |
| ≥ 5 points | 628 | 4.6 | 5167 | 2.4 | 0.004 |
| Attended screening | 595 | 4.9 | 3967 | 3.2 | 0.04 |
| Clin. DM | 29 | | 126 | | |

Opportunistic screening had higher response and attendance rates, compared to mail distributed population based screening (100% versus 49% and 95% versus 77%, respectively), but only 31% of the potential patients were offered a risk assessment in opportunistic screening.
The mean risk-score was higher and the prevalence of diabetes in the identified high-risk groups was higher in opportunistic screening compared to mail-distributed population based screening at any given level of the programme.
**Conclusions:** Opportunistic screening has higher response- and attendance rates and the participants have higher risk-scores. Despite only 31% of the patients attending practice were offered a risk-assessment, opportunistic screening identifies an equal or higher proportion of the underlying population and subsequent high-risk participants with previously unidentified DM than mail-distributed population based screening, using the same screening tools. Opportunistic screening for Type 2 DM is more effective than mail-distributed population based screening.

# OP 19

# Non-insulin analogues

## 109

**Long-acting Tyr[1]-modified analogues of GIP with significantly improved antihyperglycaemic and insulinotropic properties**

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**Background and aims:** The gut hormone glucose-dependent insulinotropic polypeptide (GIP) has shown promising effects for improving glycaemic control. However metabolic instability and rapid renal clearance limit its therapeutic potential for the treatment of type 2 diabetes. The present study examines the capacity of two novel designer fatty acid derived (FAD), N-terminally modified GIP analogues namely; *N*-pGluGIP (LysPAL$^{16}$) and *N*-pGluGIP(LysPAL$^{37}$) to resist dipeptidyl peptidase IV (DPP IV) degradation and display improved bioactivity *in vitro* and *in vivo* in *ob/ob* mice.
**Materials and methods:** GIP and related (FAD) analogues were synthesised using standard solid-phase Fmoc peptide chemistry. The stability of peptides (5 µg) was assessed by incubation (0, 2, 8 and 24 h at 37°C) in the presence of purified DPP IV (5 mU, n=3) and peptide degradation quantified by reversed-phase HPLC analysis. Cyclic-AMP production (n=6) was measured in Chinese hamster lung (CHL) fibroblast cells expressing the cloned human GIP receptor. The insulin releasing ability of GIP analogues was assessed in acute (20 min, 5.6 mmol/l glucose) studies with clonal pancreatic BRIN-BD11 cells (n=8). Effects of GIP and related analogues on plasma glucose and insulin concentrations were examined in 14- to 18-week old obese diabetic (*ob/ob*) mice (n=8) following i.p. injection (25 nmol/kg bodyweight).
**Results:** DPP IV rapidly degraded native GIP, with only 52% of the peptide remaining intact after just two hours incubation. In contrast the GIP analogues remained fully intact after an extended enzyme incubation period of 24 h. Native GIP produced a distinct dose-dependent stimulation of cAMP production ($EC_{50}$ 18.2 nmol/l). Both GIP analogues followed a similar pattern to that of native peptide with *N*-pGluGIP(LysPAL$^{16}$) and *N*-pGluGIP(LysPAL$^{37}$) having improved $EC_{50}$ values of 12.1 and 13.0 nmol/l for cAMP production, respectively. Furthermore, both (FAD) GIP analogues ($10^{-13}$ to $10^{-6}$ mol/l) demonstrated superior insulinotropic responses ($P<0.05$ to $P<0.001$) in clonal beta-cells compared with glucose alone (5.6 mmol/l). In obese diabetic (*ob/ob*) mice, administration of *N*-pGluGIP (LysPAL$^{16}$) and *N*-pGluGIP(LysPAL$^{37}$) together with glucose (18 mmol/kg bodyweight) significantly reduced the peak 60 min glucose excursion (1.3-fold; $P<0.01$ in both cases) compared with glucose alone. The area under the curve (AUC) for glucose was significantly lower after administration of either analogue compared with glucose administered alone (1.4-fold; $P<0.01$). This was associated with a significantly enhanced overall glucose-mediated insulin secretory response for *N*-pGluGIP(LysPAL$^{37}$) compared to the native peptide (1.6-fold; $P<0.01$), and in the case of *N*-pGluGIP (LysPAL$^{16}$) a significantly enhanced response when compared to glucose alone (2.0-fold; $P<0.001$). The extended action of *N*-pGluGIP(LysPAL$^{16}$) and *N*-pGluGIP(LysPAL$^{37}$) was clearly evident from 30 to 60 min with plasma insulin concentrations remaining significantly higher ($P<0.05$ to $P<0.05$ and $P<0.01$ to $P<0.05$, respectively) compared to control.
**Conclusion:** These data indicate that Tyr[1]-modification of (FAD)GIP analogues with a pyroglutamyl-group conferred stability, increased *in vitro* potency and resulted in greater insulinotropic and antihyperglycaemic activities in an animal model of type 2 diabetes.

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**A novel insulin mimetic peptide with similar pharmacodynamic action and potency as normal insulin on glucose disposal in rats**

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**Background and aims:** Small insulin-mimetic heterodimer peptides have been constructed, consisting of two peptide sequences, each binding to one of the previously discovered two insulin binding sites on the insulin receptor. These peptides have high affinity for the insulin receptor ($K_d$ in the pM range) and activate the insulin signaling pathway, as shown by increased in vitro lipogenesis and by decreased glucose levels after i.v. administration to pigs and anaesthetized rats. The aim of the current experiment was to determine the in vivo pharmacodynamic properties and potency of such an insulin mimetic peptide.
**Materials and methods:** Normal male Sprague-Dawley rats with previously implanted chronic jugular and carotid catheters were fasted overnight and injected i.v. with normal human insulin (HI; 1.25 or 2.5 nmol/kg) or with the insulin mimetic (IM; 2.5 or 5.0 nmol/kg). Plasma glucose was monitored at regular intervals to determine pharmacodynamic profile.
To assess the mimetic's potency, similarly prepared rats were fasted overnight and subjected to a hyperinsulinemic euglycemic clamp, where hyperinsulinemia was achieved by constant i.v. infusion of HI (at 15 or 480 pmol/kg·min) or IM (at 15, 30, 60, 120, 240 or 480 pmol/kg·min). Glucose was infused i.v. at a variable rate to maintain euglycemia, thus providing an index of the mimetic's potency on glucose disposal. Some of the animals were killed at the end of the experiment and muscle tissue was excised and frozen for determination of insulin receptor activation (measured by immunoprecipitation followed by determination of tyrosine phosphorylation).
**Results:** I.v. injection of IM resulted in a glucose lowering profile similar to that obtained with HI. An approximately two times higher dose of IM was needed to decrease glucose levels to the same extent as HI (curve area 193 ± 23 and 281 ± 21 mM·min for 1.25 and 2.5 nmol/kg HI, and 221 ± 32 and 303 ± 13 mM·min for 2.5 and 5.0 nmol/kg IM; average ± SEM).
During constant i.v. infusion of IM, the glucose infusion rate (GIR) required to maintain euglycemia showed a clear dose-dependency (steady-state GIR levels 4 ± 1, 15 ± 2, 24 ± 1, 30 ± 2, 31 ± 1 and 38 ± 1 mg/kg·min for the respective dosages of IM; Pearson correlation $p<0.05$). At low dosage, infusion of IM resulted in lower GIR compared to HI (at 15 pmol/kg·min, GIR was 4 ± 1 for IM vs 17 ± 1 mg/kg·min for HI; $p<0.05$), but at the high dosage both compounds resulted in similar GIR (38 ± 1 vs 35 ± 1 mg/kg · min, at 480 pmol/kg·min). The findings were confirmed by increased insulin receptor activation after IM infusions, to a similar or higher degree compared to HI (fraction of phosphorylated receptors, expressed relative towards an insulin-infused rat: 1.30 ± 0.17, 1.44 ± 0.32, and 1.96 ± 0.29* for 30, 60 and 120 pmol/kg·min IM; *$p<0.05$ vs HI).
**Conclusion:** We have engineered a small insulin mimetic peptide which has high affinity for the insulin receptor, stimulates the insulin signaling pathway, and decreases blood glucose similar to normal insulin. Steady-state euglycemic clamp conditions show that it dose-dependently increases whole-body glucose disposal, with a potency in the same range as normal insulin. Therefore, these small insulin mimetic peptides offer a promising new approach to the treatment of diabetes.

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**Effects of pramlintide on postprandial glucose excursions and measures of oxidative stress in patients with Type 1 diabetes**

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**Background and aims:** Oxidative stress (OS) has been shown to be increased in the postprandial (PP) period in patients with diabetes (DM), and has been implicated in the pathogenesis of micro- and macrovascular complications. This study assessed the effects of pramlintide (PRAM), an analog of the naturally occurring β-cell hormone amylin, on markers of OS in the PP period.
**Materials and methods:** In a randomized, single-blind, placebo-controlled, crossover study, 18 subjects with type 1 DM (age 38 ± 15 y, duration of DM 22 ± 12 y, $HbA_{1c}$ 9.4 ± 1.7% [mean±SD]) underwent 2 standardized breakfast meal tests, t=0 min. In addition to their preprandial injection of regular insulin (7.2 ± 0.9 and 7.5 ± 1.0 U [mean±SE] at t=−30 min for PRAM and placebo [PBO], respectively), subjects received a subcutaneous injection of either PRAM (60 µg at t=0 min) or PBO (at t=-15 min). The plasma concentrations of glucose and markers of OS (nitrotyrosine [NT], oxidized LDL cholesterol [oxLDL-C], and total-trapping antioxidant parameter [TRAP]) were assessed at baseline and during the 4-h PP period.
**Results:** Compared with PBO, PRAM significantly reduced PP excursions of glucose (>100%), NT (>100%) and oxLDL-C (>100%), and protected TRAP from consumption (>100% increase). Correlation analyses adjusted for treatment revealed a significant association between change in glucose and change in each measure of OS ($P<0.001$ for all correlations). The most frequent adverse events were mild to moderate hypoglycemia and mild nausea.
**Conclusion:** In summary, the reduction in PP glucose excursions achieved with the addition of PRAM to short-acting insulin in type 1 DM was associated with a significant reduction in PP oxidative stress.

| PP Excursions (incremental $AUC_{0-4h}$) | PRAM | PBO | *P*-value |
|---|---|---|---|
| Glucose (mmol/L · h) | −0.6 ± 2.5 | +11.0 ± 2.9 | 0.001 |
| NT (μmol/L · h) | 0.07 ± 1.4 | 5.3 ± 2.2 | 0.014 |
| OxLDL-C (U/L · h) | −4.3 ± 3.4 | 19.4 ± 6.0 | 0.002 |
| TRAP (μmol/L · h) | −0.79 ± 73.3 | −200 ± 89.4 | 0.021 |

Mean ± SE, *P*-values based on mixed-effects models.

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**Safety and tolerability of long-term pramlintide therapy**

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**Background and aims:**
The DCCT and UKPDS demonstrated the benefits of intensified diabetes therapy and that long-term intensive therapy is difficult for patients to maintain due to increased risk of severe hypoglycemia and weight gain. Pramlintide (PRAM), a synthetic amylin analog, has been studied as an adjunct to insulin therapy in 26- and 52-week clinical trials since 1992. We will present data on 16 subjects from our site who received PRAM for > 2years as participants in an open-label, safety and tolerability study.
**Materials and methods:** In this open-label study, PRAM as an adjunct to insulin was administered subcutaneously by a separate injection with meals. PRAM was initiated with dose titration and insulin adjustments to help prevent severe hypoglycemia and reduce nausea. Our research site has enrolled 27 subjects:16 have received PRAM for >2 years , 4 have been on PRAM <2 years, and 7 withdrew from the study (3 withdrew consent, 2 moved away, and 2 planned pregnancies). 22 of the 27 had participated in prior randomized pramlintide trials, but were not receiving PRAM at the time of entry into this open-label study. We present data on the 16 subects who had >2 y exposure to PRAM: 13 type 1 and 3 type 2, mean age 48 y (range 30–75 y), mean BMI 27 kg/m$^2$, median 25 (range 22-40 kg/m$^2$), mean A1C 7.6%, 7.5% median (range 5.8–10.1%), 38% of subjects had an A1C ≤7.0%, and mean disease duration 22 y (range 3-41 y). Of the 16 subjects, 11 were treated with CSII and 5 with basal-bolus insulin analog therapy.
**Results:** The mean PRAM exposure was 4 y (range 2-7 y). Seven subjects withdrew from the study. At follow-up, the mean total daily PRAM dose was 194 μg (range 72-270 μg), the mean A1C was 7.4%, with a 7.0% median (range 5.9-10.2), and a mean BMI of 26.4 kg/m$^2$ median 25 kg/m$^2$ (22–39 kg/m$^2$). Subjects with baseline BMI >25 lost a mean of 9 lbs. at follow-up. Prior to study entry, 6 subjects had reported episodes of severe hypoglycemia. Severe hypoglycemia was not reported during PRAM initiation. During maintenance, 2 subjects had 1 episode each of severe hypoglycemia on PRAM (12.5%), an incidence of 3.1 episodes/100 pt-years. Vomiting was not reported and no subject discontinued PRAM due to nausea. With regard to nausea, 9 subjects (56%) reported no nausea on PRAM, while 7 subjects reported mild, intermittent nausea of mean duration 22 days (range 3–61 days) occurring with PRAM initiation or dose titration.
**Conclusion:** PRAM was well-tolerated and resulted in increased numbers of subjects reaching the ADA goal of A1C ≤7.0%. Despite the necessity of additional daily injections, subjects continued to use PRAM. Subjects were able to maintain intensified therapy with a reduction in severe hypoglycemia and without weight gain.
*Supported by: Amylin Pharmaceuticals*

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**Dipeptidyl peptidase IV (DP-IV) inhibition for the treatment of Type 2 diabetes: potential importance of selective inhibition and discovery of MK-0431**

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**Background and aims:** DP-IV inhibitors are a new approach to type 2 diabetes that lower glucose via stabilization of the peptide hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), which have clearly established roles in glucose-dependent insulin secretion. DP-IV is a member of an emerging group of serine dipeptidases that includes QPP, DPP8, and DPP9. A number of functions beyond incretin processing have been suggested for DP-IV, including T cell activation and neuropeptide regulation. The aim of this work was to address issues that relate to DP-IV inhibition as a treatment for diabetes including: i) glycemic efficacy, ii) potential effects on beta cell function, and iii) potential safety/tolerability issues due to nonselective inhibition and other reported functions for DP-IV.
**Materials and methods:** We identified highly selective inhibitors of DP-IV, QPP, DPP8/DPP9 and evaluated these compounds in models of diabetes, immune function and in preclinical toxicity studies.
**Results:** The DPP8/9 inhibitor attenuated T cell proliferation in *in vitro* models of immune function, and produced profound toxicities in rats (alopecia, thrombocytopenia, anemia, enlarged spleen, multiple histological pathologies, and mortality) and dogs (bloody diarrhea, emesis, and tenesmus). In contrast, no effects were observed with DP-IV and QPP selective inhibitors in these models. Based on these findings, we focused on the discovery of selective DP-IV inhibitors. These efforts led to the identification of a novel β-amino acid derived inhibitor, MK-0431, which is a potent inhibitor of DP-IV ($K_i$ = 9 nM) that is highly selective over all proteases tested ($IC_{50}$s > 50 μM), including QPP, DPP8, and DPP9. In lean mice, MK-0431 increased circulating active GLP-1, and reduced blood glucose excursion following an OGTT (55% at 3 mg/kg PO). In mice with diet-induced obesity, a dose dependent (0.3, 3, 30 mg/kg PO) decrease in blood glucose excursion was observed following oral glucose administration. A close structural analog of MK-0431 was administered to HFD-STZ mice for 3 months and found to decrease postprandial and fasting hyperglycemia; near normalization of the insulin/glucagon ratio was observe in islets from the treated mice. MK-0431 has excellent oral bioavailability in mice, rats, dogs, and monkeys (61, 76, 100, and 68%, respectively), and a half-life ranging from 1–5 hours.
**Conclusions:** These results strongly suggest that DP-IV activity is not important for T cell activation and that selective inhibition of DP-IV may be required for an acceptable safety and tolerability profile for this class of antihyperglycemic agents. MK-0431 is a highly selective DP-IV inhibitor currently in clinical development that has a promising profile in this class. Results with this and related compounds in animal models of diabetes suggest that DP-IV inhibition will be a safe and effective treatment for type 2 diabetes with the potential to preserve or enhance beta cell function.

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**Efficacy and two-year pulmonary safety of inhaled insulin as adjunctive therapy with metformin or glibenclamide in Type 2 diabetes patients poorly controlled with oral monotherapy**

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**Background and aims**: Inhaled insulin offers an alternative to oral agents or injected insulin in patients with poorly controlled type 2 diabetes. The efficacy and 2-year safety of adjunctive therapy with inhaled insulin (INH, Exubera®) or an additional oral agent (OA) was examined in 2 studies in patients with type 2 diabetes, poorly controlled by oral monotherapy ($HbA_{1c} \geq 8\%$). The primary objective of the 2-year study was to assess the long-term pulmonary safety of INH use.
**Materials and methods**: Both studies were open-label, 24-week studies extended to 104 weeks. Patients receiving metformin (Study 1) or a sulphonylurea (Study 2) were randomized to either adjunctive INH (n = 471) or an additional OA [metformin or glibenclamide (n = 441)]. Additional concomitant diabetic therapies were allowed during the extension period. A washout evaluation of pulmonary function was carried out 6 and 12 weeks post-discontinuation.
**Results:** 158 patients on INH, and 146 on OA completed the 104-week extensions of these 2 studies. Demographic characteristics, changes in $HbA_{1c}$, pulmonary function tests, and adverse event rates were comparable between those who entered the extension and those who did not. For the completers, the mean daily INH dose was 13.8 mg at Week 24 and 16.8 mg at Week 104. Mean baseline $HbA_{1c}$ was 9.6% in both groups, decreasing to 7.7 ± 1.4% (INH) and 8.1 ± 1.3% (OA) at Week 104. There were no severe hypoglycemic events and the overall rate of hypoglycemia was lower in the INH group than the OA group [INH/OA risk ratio=0.81 (95% CI: 0.71, 0.92)]. The most common respiratory adverse event was cough, which was considered to be transient and mild. At Week 24, decreases in $FEV_1$ were larger with INH than OA, but the adjusted difference between groups decreased after Week 36 (Table 1). Differences in $DL_{CO}$ between groups were small, relative to the standard deviations of the measurement (Table 1). There was no discernable treatment group difference in either lung function endpoints 12 weeks after discontinuing 2 years of therapy (Table 1).

**Conclusion:** Inhaled insulin (Exubera®) is effective and well tolerated over 2 years as an adjunctive therapy in patients with type 2 diabetes poorly controlled by oral monotherapy. Treatment group differences in pulmonary function are small, occur early after treatment initiation, have no identified clinical relevance, and do not progress with up to 2 years of continued INH treatment. Treatment effects are reversible within 12 weeks post discontinuation.

**Table 1.** Treatment group differences over time for change from baseline in forced expiratory volume in one second ($FEV_1$) (L/sec) and carbon monoxide diffusing capacity ($DL_{CO}$) (mL/min/mm Hg)

| | Time (weeks) | INH | OA | Adjusted difference* (INH – OA) (95% CI) |
|---|---|---|---|---|
| FEV1 | 24 | −0.060 | +0.008 | −0.063 ± 0.025 (−0.111, −0.014) |
| (L/sec) | 36 | −0.063 | −0.052 | −0.007 ± 0.025 (−0.057, +0.043) |
| | 52 | −0.093 | −0.070 | −0.019 ± 0.025 (−0.067, +0.030) |
| | 104 | −0.170 | −0.128 | −0.039 ± 0.028 (−0.093, +0.015) |
| | +6** | −0.142 | −0.133 | +0.004 ± 0.028 (−0.058, +0.051) |
| | +12** | −0.158 | −0.136 | +0.014 ± 0.027 (−0.066, +0.039) |
| DLCO | 24 | −0.688 | −0.281 | −0.275 ± 0.371 (−1.002, +0.452) |
| (mL/min/ | 52 | −1.172 | −0.808 | −0.260 ± 0.393 (−1.030, +0.510) |
| mm Hg) | 104 | −1.529 | −1.583 | +0.112 ± 0.392 (−0.655, +0.880) |
| | +6** | −1.096 | −1.368 | +0.279 ± 0.383 (−0.473, +1.030) |
| | +12** | −1.304 | −1.072 | −0.084 ± 0.410 (−0.888, +0.721) |

* A negative value indicates a lesser increase or a greater decrease in the INH than comparator group.
** Post discontinuation

# OP 20

# Mechanisms in cardiac complications

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**Role of nitric oxide in regulation of extracellular superoxide dismutase in diabetes mellitus**

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**Background and aims:** The extracellular superoxide dismutase (ecSOD) has been reported as one of the major antioxidant enzyme system in the arterial wall. ecSOD supports the biologic activity and stability of nitric oxide (NO) which is essential for regulation of vasoreactivity but can also react with ROS (reactive oxygen species) to peroxynitrite. Previously we have shown that endothelial function is impaired by inhibition of SOD, an effect which is more distinct in diabetic than in control rats. We also found that its activity is reduced in diabetic rats compared to controls. However, no details are known about the mechanisms determining the synthesis of ecSOD and its release in diabetes.
**Materials and methods:** To identify in which vascular cells ecSOD is synthesized we applied RT-PCR technique (reverse transcription-polymerase chain reaction) with specific primers for ecSOD. To determine ecSOD activity a biochemical colorimetric assay based on the oxidation of nitrotetrazolium blue (NBT) was used.
**Results:** mRNA encoding the ecSOD protein was detected only in smooth muscle cells of rat thoracic aorta (RASMC), but not in endothelial cells. The activity in RASMC was low as compared to that in supernatant indicating that the enzyme is less stored in the cells but mostly secreted from smooth muscle cells into the medium: 0,315 ± 0,048 $U^*min^{-1}*ng^{-1}DNA$ (medium) vs. 0,08 ± 0,01 $U^*min^{-1}*ng^{-1}DNA$ (cell lysate). Incubating RASMC with the NO-donor SNAP (S-nitroso-amino-penicillin) for 96 hours caused a dose dependent increase of ecSOD in the medium. Using 200 µM SNAP ecSOD activity was elevated up to 14-fold compared to control conditions: 11,957 ± 2,804 $U^*min^{-1}$ vs. 0,824 ± 0,159 $U^*min^{-1}$. An increase of glucose in the medium from 5 mM to 30 mM (for 96 hrs.) also caused an elevation in ecSOD activity to the medium of RASMC: 0,824 ± 0,159 $U^*min^{-1}$ (5 mM) vs. 3,428 ± 0,648 $U^*min^{-1}$ (30 mM).
**Conclusion:** We conclude that ecSOD is synthesized preferentially by smooth muscle cells and is released by these cells. Endothelial cells do not contribute to the synthesis, but ecSOD is adsorbed at their surface. If cells are incubated with an NO-donor (e.g. SNAP) ecSOD activity is enhanced indicating that NO up-regulates ecSOD. Further more upregulation of ecSOD is also observed in acute hyperglycemia. Based on these findings we assume that ecSOD is upregulated in hyperglycemia presumably by induction of NO-synthase. Such mechanism is helpful to reduce oxidative stress associated with hyperglycemia to increase the bioavailability of nitric oxide and thereby maintain endothelial function.

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**Possible mechanisms of insulin participation in pathogenesis of heart deteriorations in diabetes mellitus**

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**Background:** It was recently shown that insulin (Ins) attenuates adrenergic and cholinergic coronary artery (CA) dilation. Nevertheless exact mechanisms of heart function disorders induced by insulin resistance and hyperinsulinemia in diabetes mellitus remain largely unknown.
**Aim:** The study was devoted to investigation of possible pathogenetic mechanisms of cardiovascular alterations in experimental model of acute hyperinsulinemia.
**Materials and Methods:** Experiments on 32 healthy dogs under chloralose anesthesia (30–100 mg/kg, i.v.) were performed. Catetherization, extracorporal programmed autoperfusion of circumflex CA with constant volume of arterial blood, heart and main vessels catetherization, catetherization 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 registrated.
**Results:** In model experiments with acute hyperinsulinemia induced by Ins administration (1.0 IU/kg, i.v.), during first 5-10 min (1 phase) CA resistance increased. Its magnitude was determined by the degree of CA perfu-

sion pressure elevation (+1.4 ± 0.4 kPa; p<0.001). During this phase $+dP/dt_{max}$ and $-dP/dt_{max}$ increased (+25.8 ± 5.8 and +24.5 ± 8.4 kPa/s; p<0.01, resp.), femoral artery perfusion pressure decreased (-2.8 ± 0.7 kPa; p<0.01). Due to heart function elevation the myocardial oxygen and energetic substrates demands augmented (coronary artery-venous difference by oxygen increased on 8.4 ± 2.8%; p<0.01). During next 30–60 min after Ins injection (2 phase) reductions of CA perfusion pressure (-1.8 ± 0.3 kPa; p<0.001), both $+dP/dt_{max}$ and $-dP/dt_{max}$ (-40.5 ± 12.8 and -52.0 ± 18.1 kPa/s; p<0.001, resp.) and decrease of coronary artery-venous difference by oxygen (-10.4 ± 1.6%; p<0.001) took place. After blockades of NO-synthase activity (L-NAME, 1.0 mg/min intracoronary infusion), $Na^+,K^+$-ATPase activity (ouabaine, 100 mg, i.v.) and guanilatcyclase activity (methylene blue, 1.0 mg/min, intracoronary infusion) CA dilation disappeared and only its constriction was observed. After beta-adrenoreceptors blockade (propranolol, 2.0 mg/kg, i.v.) Ins induced mainly CA constriction which was accompanied by significant contractility heart function elevation. Increase of adrenergic system activity after M-cholinergic receptors blockade (atropine, 0.5 mg/kg, i.v.) limited or absolutely abolished CA dilation and changes of most cardiohaemodynamic effects on Ins injection.
**Conclusion:** We suggest that hyperinsulinemia can directly take part in pathogenesis of diabetic cardio-haemodynamic disturbances especially in the presence of endothelial dysfunction.

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**Myocardial infarct size attenuation by glucagon like peptide-1 (GLP-1) in both in vivo and in vitro rat heart**

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**Background and aims:** *Glucagon-like peptide-1 (GLP-1)* is a gut incretin hormone which stimulates postprandial insulin secretion. Insulin has been shown to reduce cell death in the ischaemic-reperfused rat myocardium and in isolated rat myocytes via its ability to activate prosurvival kinase signalling pathways.On the other hand, GLP-1 has been shown to have anti-apoptotic effects in pancreatic and insulinoma cell lines. Therefore, we propose that GLP-1 could also protect the myocardium against ischaemia/reperfusion injury by either a direct effect or via insulin activating similar prosurvival signalling pathways.
**Materials and methods:** Both in vivo (open chest) and in vitro (isolated Langendorff perfused) rat heart models of regional ischaemia (30 mins) and reperfusion (120 mins) were used, with myocardial infarct size being the end point studied. Infarct size was measured using triphenyl-tetrazolium chloride staining and expressed as a percentage of the area at risk (I/R%) GLP-1 (4.8 pmol/kg/min) was given in vivo as an infusion intravenously, whilst in vitro 0.3nmol GLP-1 was added, to the buffer, during stabilisation and continued throughout the experiment protocol. In order to assess the role of intact GLP-1, which is rapidly degraded into inactive fragments by dipeptidyl peptidase-IV (DPP-IV) to give GLP-1 a half life of minutes, an inhibitor of DPP-IV namely, valine pyrrolidide (VP) 20 mg/kg in vivo and 20 mg/L in vitro was used. In vitro experiments were performed using the GLP-1 receptor antagonist exendin-9-39 3nM, the PKA antagonist Rp-cAMP 1.5uM and inhibitors of known prosurvival pathways such as LY 24009 15uM (which inhibits PI3Kinase) and UO 126 10uM (which inhibits p42/44 MAPK). These drugs were added to the perfusate during stabilisation of the hearts and continued thruoghout ischaemia and reperfusion. In vivo and in vitro haemodynamic data were recorded.
**Results:** The in vivo hearts treated with GLP-1 demonstrated a significant reduction in infarction (% infarct/risk zone) compared to the VP and saline control groups (20.0+/-2.8, vs. 47.3 +/-4.3, and 44.3+/-2.4, respectively p<0.001). Interestingly, in the isolated perfused Langendorff rat hearts (where there is no circulating insulin) the GLP-1 also significantly reduced infarct size compared to VP and saline control (26.7+/-2.7 vs. 52.6+/-4.7 and 58.7+/-4.1, p<0/001) groups respectively. Additionally, this protection was abolished in the presence of the PI3 kinase inhibitor, LY294002 (58.6+/-4.1), and the p42/44 MAPK inhibitor, UO126 (48.3+/-8.6), implicating both these pro survival pathways in the cardio-protection mediated by GLP-1. Protection was also abolished by the GLP-1 receptor antagonist exendin-9-39 (57.3+/-3.8) and by Rp-cAMP (57.5+/-5.0).There were no significant haemodynamic effects of GLP-1.
**Conclusion:** Our data have shown, for the first time, that GLP-1 is able to protect the myocardium against ischaemia/reperfusion injury in both the in vivo and in vitro rat heart. This action of GLP-1 is transduced through the GLP-1 receptor. GLP-1 appears to act by the up regulation of specific prosurvival kinase pathways involving PI3K, PKA and p42/44 MAPK. This may represent a new therapeutic potential for this class of drugs currently undergoing trials in the treatment of non-insulin dependent diabetes.

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**Association between collagen Type I Alpha 1 Sp1 (COLIA1) polymorphism and severity of coronary heart disease in Type 2 diabetes.**

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**Background and aims:** Collagen is one of the most important extracellular matrix proteins and plays an essential role both in physiologic and pathophysiologic processes. The collagen proteins are highly involved in the pathogenesis of atherosclerosis, persuading the stability of the atheroma's fibrous cap and interacting with macrophages, smooth muscle cells and platelets. The COLIA1 +2046G>T polymorphism - taking effect on collagen quality - has been associated with osteoporosis so far, but it is possible that there is also an association to atherosclerosis due to collagen's key role. In this study we investigated the association between COLIA1 genotype and the severity of coronary heart disease - as the major complication of atherosclerosis - in type 2 diabetes.
**Materials and methods:** One hundred and eleven type 2 diabetic patients and a control group of one hundred and fifty-six non-diabetic patients undergoing coronary angiography were studied. The COLIA1 genotype was assessed using polymerase chain reaction followed by *Bal*I digestion. Coronary heart disease was defined by angiographic criteria, the severity of coronary heart disease by the number of coronary arteries with ≥50% lumen narrowing: A 3-vessel-disease corresponded to severe CHD, whereas 2-, 1-vessel-disease or the absence of any relevant stenosis corresponded to minor CHD.
**Results:** The COLIA1 genotypes were distributed as follows: 66,7% GG, 32,4% GT, 0,9% TT in type 2 diabetic patients and 73,1% GG, 25% GT and 1,9% TT in the control group. The comparison between the COLIA1 genotypes and the severity of coronary heart disease showed the following results: Type 2 diabetic patients with genotypes GT/TT (n=37) suffered significantly more often from severe coronary heart disease than diabetic patients with genotype GG (n=74) (p=0,042). In the control group there was no association between COLIA1 genotypes and coronary heart disease.
**Conclusions:** The COLIA1 gene polymorphism is associated with the severity of coronary heart disease in type 2 diabetes. Changes of the collagen matrix at the molecular level may take effect on atherosclerosis: An overproduction of collagen by fibroblasts resident in atheroma and an altered interaction between collagen and smooth muscle cells and macrophages followed by increased proliferation may be pro-atherogenic mechanisms. Further, the altered collagen fibrillar structure may lead to plaque weakness and vulnerability and make it so prone to rupture.

## 119

**Myocardial nerve growth factor levels: effects on vascularisation and innervation in patients with diabetes and ischaemic heart disease**

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**Background and aims:** Abnormalities in myocardial vascularisation ands innervation may lead to silent myocardial ischaemia and arrythmias. Nerve growth factor (NGF) maintains both vascular and neuronal phenotype.
**Materials and methods:** The right atrial appendage was removed to establish cardiopulmonary bypass in: patients undergoing: valve replacement without ischaemic heart disease (C) (n=3); and in non-diabetic (IHD) (n=8) and diabetic (D) (n=5) patients undergoing coronary artery bypass grafting. NGF (pg/mg tissue) was measured in extracts of atrial appendages using anti-human NGF.
**Results:** It was significantly reduced in patients with IHD (0.11 + 0.02), (p=0.002), compared to C (0.25 + 0.05). Diabetic patients demonstrated the most significant reduction (0.07 + 0.02) (P=0.0001). The density of small nerve fibres (SNF's) was established in relation to cardiomyocyte loss in an additional 50 patients (C- n= 13, IHD- n= 17, D with IHD-n= 15, D without IHD n= 5) using immunohistochemical labelling of neural fibres with antibody to the PGP9.5. The SNF density was increased in those with IHD 458.3 + 48.6 and reduced in D without IHD- 167.8 + -43.9 but increased in D with IHD-339.9 + 58.8 though it was still less than C-379.3 + 55.2. In diabetic patients SNF's survived at the periphery of cardiomyocyte foci but were lost in the middle of larger muscle cell groups and the intensity of their staining was more pronounced than in control cases. There was also loss of subendocardial nerve fibres in the diabetic group. To define the effect on cardiomyocytes, selected sections were counterstained with fluorescent nuclear dye, and the ratio of nerve fibres:cardiomyocyte nuclei was increased for D with IHD (n=3, ratio 1.32) v D without IHD (n=3; ratio

1.16). indicating a compromised balance between remaining cardiomyocytes and their SNF supply. To further understand the relation between myocardial innervation and vascularization the area of endothelium stained for endothelial cell markers/mm2 and area of nerve fibres stained for PGP9.5 were assessed and the ratio of endothelial:nerve staining calculated. The lowest ratio 3.5+/-1.3 was in the control group (C), followed by IHD 4.1+/-1.5, diabetics without IHD 6.75+/-1.4 and diabetics with IHD 11.2+/-7.5. The differences in BV/N ratio between D with IHD and controls was highly significant ($p<0.001$), D with IHD and IHD alone was also significant ($p<0.01$).
**Conclusion:** The reduction in NGF levels may provide a molecular basis for the alteration in myocardial vascularisation and innervation in patients with diabetes and IHD. Diabetes alone appears to result in a reduction in atrial innervation, however IHD appears to be promote reinnervation and neovascularisation, presumable by factors other than NGF.

## 120

**Acarbose reduces silent myocardial infarctions in patients with impaired glucose tolerance. Results of the randomized STOP-NIDDM ECG substudy**
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**Background.** The moderate increase in post-prandial plasma glucose in subjects with impaired glucose tolerance has been shown to be a predictor of cardiovascular disease. In the randomized STOP-NIDDM Trial, we could demonstrate that lowering post-prandial plasma glucose with acarbose in subjects with impaired oral glucose tolerance could reduce the risk of diabetes.
**Methods:** In the STOP-NIDDM trial subjects aged between 40 and 70 years with impaired glucose tolerance were randomised to placebo or acarbose 100 mg 3xdaily. ECG were obtained at baseline and at the end of of the double-blind study treatment. The mean follow-up was 3.3 years. The current report focuses on the effect of acarbose on silent ischemic events evaluated in the electrocardiographic substudy, using the Minnesota code classification
**Results:** A total of 1181 patients were included in the ECG substudy. From these 72 patients had significant changes between the baseline and end of treatment ECG, 33 in the acarbose and 39 placebo group (Table 1). Higher rates of myocardial infarctions occurred in the placebo group ($p=0.07$ with Fisher`s Exact test and $p=0.023$ with Chi-square test), while there no differences between the two groups with ECG changes classified to the other Minnesota codes. In addition clinically overt myocardial infarctions occurred in 1 patient in the acarbose and 12 patients in the placebo group (Table 2).
**Conclusions:** In this prospective intervention study we could show, that acarbose by decreasing post-prandial hyperglycemia can reduce the incidence of silent and clinically overt myocardial infarctions in subjects with IGT. This approach should therefore evaluated in other higher risk populations.

ECG changes between baseline and end of treatment

| | Placebo (n=604) | Acarbose (n=577) |
|---|---|---|
| Myocardial infarction | 1.2% | 0.2% |
| Elevated wave amplitudes | 0 | 0.2% |
| ST dpression and T wave negativity | 4.0% | 3.8% |
| A-V conduction defect | 0 | 0.2% |
| complete bundle branch block | 0.9% | 0.9% |
| Arrhythmias | 0.6% | 0.6% |

Myocardial infarctions in STOP-NIDDM

| | Placebo (n=686) | Acarbose (n=682) | p-value |
|---|---|---|---|
| Clinically overt | 12 | 1 | 0.02 |
| Silent | 7 | 1 | 0.07 |
| Total | 19 | 2 | < 0.01 |

*Supported by: Bayer-Vital AG*

# OP 21

# Risk factors – microvascular complications

## 121

**Risk factors for neuropathy in UKPDS**
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**Background and Aims:** Neuropathy is a major source of morbidity in subjects with Type 2 diabetes. We have sought to quantify this problem and examine risk factors measured at diagnosis for incident and prevalent neuropathy.
**Materials and Methods:** UK Prospective Diabetes Study recruited 5102 subjects with newly diagnosed type 2 diabetes who were followed for up to 25 years. Data on neuropathic indices were collected at entry to the study and at three year intervals. Incident cases were defined as those with evidence of neuropathy events recorded at two consecutive visits up to 12 years post diagnosis, in those without events initially. Prevalence was analysed using logistic regression and incidence with Cox proportional hazards models. HbA1 c was taken at entry for the prevalence analyses and after 3 months dietary intervention for newly incident cases. Other data was collected at entry.
**Results:** Prevalence and incidence at 12 years of reduced great toe perception threshold (VPT) > 25V, absent ankle jerks(AJ) bilaterally, and self-reported erectile dysfunction (ED) in males are shown in the table. The hazard ratios for age (per 5 years), female sex, HbA1 c (per 1%), height (per 5 cm), waist circumference (per 5 cm),regular or heavy alcohol consumption, current smoking and weight (per 5 kg) are shown where statistically significant. Risk factors within each index for incidence and prevalence are similar. At 12 years 64% of men and 44% of women free of neuropathy at baseline were found to be positive for at least one of these indices.
**Conclusions:** Many of those newly diagnosed with Type 2 diabetes, 36% of men and 21% of women, already have evidence of neuropathy. Taller subjects are at greater risk, as are those who are older at diagnosis. Regular alcohol consumption is associated with the risk of incident and prevalent impotence in men. By 12 years from diagnosis 71% of men and 51% of women have clinically significant neuropathy.

Risk factors for neuropathy prevalence at diagnosis and incidence by 12 years

| | Proportion | Age | Female | HbA1 c | Height | Waist | Alcohol | Current smoker | Weight |
|---|---|---|---|---|---|---|---|---|---|
| VPT >25 -Prevalence | 12.8% | 1.89 (1.73 to 2.07) | | (1.32 to | 1.40 (1.01 to 1.50) | 1.05 1.10) | | | |
| -Incidence | 13.3% | 1.58 (1.44 to 1.73) | (1.02 to | 1.10 (1.14 to 1.17) | 1.22 1.31) | | (1.00 to | 1.29 (1.04 to 1.67) | 1.08 1.13) |
| AJ absent -Prevalence | 14.5% | 1.28 (1.20 to 1.37) | 2.09 (1.57 to 2.80) | 1.06 (1.01 to 1.11) | 1.17 (1.09 to 1.26) | 1.14 (1.10 to 1.19) | | | |
| -Incidence | 22.2% | 1.11 (1.05 to 1.18) | | 1.13 (1.08 to 1.19) | | 1.11 (1.08 to 1.15) | | | |
| ED-Prevalence | 20.4% | 1.41 (1.28 to 1.58) | N/A | | | | 2.00 (1.59 to 2.52) | | |
| -Incidence | 34.3% | 1.19 (1.12 to 1.26) | N/A | | 1.11 (1.05 to 1.17) | | 1.27 (1.03 to 1.56) | | 1.05 (1.01 to 1.08) |

## 122

**Cardiovascular risk profile and micro- and macrovascular complications in smoking Type 1 diabetic patients**

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**Background and aims:** In the general population, smoking is associated with an increased risk of morbidity and mortality, predominantly due to cardiovascular disease. Although many studies have found an increased risk of late complications in type 1 diabetic smokers, data from large-scale studies remains scarce. The aim of the present study was to further define the cardiovascular risk profile in the type 1 diabetic smoker and to address the impact of smoking on the rate of development of late complications.
**Materials and methods:** All 3275 type 1 diabetic patients participating in the nation-wide FinnDiane Study with data on smoking habits were included. The association between smoking and other cardiovascular risk factors (obesity, waist-hip ratio, blood pressure, glycemic control, lipids) was assessed. The impact of smoking on micro- and macrovascular complications was assessed with Kaplan-Meier survival analysis stratified for sex with diabetic nephropathy, end-stage renal disease (ESRD), retinopathy requiring laser photocoagulation, and a combined macrovascular endpoint as outcome variable.
**Results:**

(a) unadjusted $P<0.05$; (b) age-, sex-adjusted $P<0.05$

| Variable | Non-smokers | Smokers | Ex-smokers |
|---|---|---|---|
| n | 1790 | 784 | 701 |
| Males (%) | 46 | 55[a] | 57[a] |
| Age (yrs) | 37 | 36 ± 10 | 41 ± 11[a] |
| Waist-hip ratio | 0.856 ± 0.084 | 0.871 ± 0.083[b] | 0.887 ± 0.088[b] |
| Systolic BP (mmHg) | 133 ± 18 | 131 ± 18[b] | 137 ± 19[b] |
| Diastolic BP (mmHg) | 79 ± 10 | 79 ± 10 | 81 ± 10[b] |
| HbA1 c (%) | 8.3 ± 1.5 | 8.8 ± 1.5[b] | 8.4 ± 1.5[b] |
| Daily insulin dose (IU/kg) | 0.71 ± 0.23 | 0.77 ± 0.25[b] | 0.69 ± 0.26 |
| Total cholesterol (mmol/l) | 4.87 ± 0.97 | 5.03 ± 1.00[b] | 5.10 ± 1.00[b] |
| LDL cholesterol (mmol/l) | 2.76 ± 0.82 | 2.87 ± 0.96[b] | 2.94 ± 0.89[b] |
| Triglycerides (mmol/l) | 1.07 ± 0.66 | 1.33 ± 0.85[b] | 1.27 ± 0.88[b] |

Survival free from diabetic nephropathy and retinopathy was profoundly impaired in smokers (present smokers and ex-smokers combined) compared to non-smokers in both males and females (log rank test; $P<0.001$) as was survival free from macrovascular disease in males ($P<0.05$). Survival free from ESRD was not affected.
**Conclusion:** Smoking is not an isolated cardiovascular risk factor in type 1 diabetes. On the contrary, smokers present with a cluster of modifiable risk factors for micro- and macrovascular complications, such as abdominal obesity, poor glycemic control, elevated daily insulin dose and dyslipidemia. As a consequence, smoking is strongly associated with micro- and macrovascular complications. More emphasis should be put on smoking cessation in the care of the type 1 diabetic patient.

## 123

**Increased risk for diabetic nephropathy in smoking patients carrying a polymorphism in the endothelial cell nitric oxide synthase gene**

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**Background and aims:** The development of diabetic nephropathy (DN) depends on poor metabolic control and lifestyle related factors such as smoking, but genetic factors are also involved. An elevated glomerular filtration rate is suggested to be involved in the pathogenesis of DN. Nitric oxide is a vasodilating molecule that is important in the control of blood flow in the glomeruli. It is produced by endothelial cell nitric oxide synthase (ecNOS) in the blood vessels and nicotine exposure in vitro can induce the expression of ecNOS. The aim of this study was to investigate if two known genetic polymorphisms in the ecNOS are associated with increased risk for developing DN, and if any of these show interaction with smoking.
**Materials and methods:** Patients with diabetes duration of 20 years or more, without albuminuria, were considered as controls (n=200). Albumin excretion rate 20–200 µg/min was considered as incipient nephropathy (n=74) and albumin excretion rate ≥200 µg/min was considered as overt nephropathy (n=48). DNA samples were collected and smoking habits were obtained from questionnaires. The genetic markers were a four/five 27 bp repeat in intron 4 and a SNP that causes a Glu-Asp exchange in exon 7.
**Results:** Patients that were smokers or ex-smokers and carrying the four repeats a-allele of the ecNOS gene were at higher risk of developing any kind of DN than non-carriers, OR=2.99 (95%=1.36–6.59) the OR for developing overt DN was 5.03 (95% CI=1.89–13.38). Carriers that were ex-smokers had 4.5 times higher risk of developing overt nephropathy. Present smoking increased the risk for DN six to seven times in a-allele carriers. When adjusting for duration of diabetes, HbA1 c, MAP and sex, the effect of the ecNOS4a allele was statistically significant for development of overt DN. A multiplicative interaction term between smoking and the ecNOS4a-polymorphism was statistically significant. No association was found for the amino acid changing polymorphism in exon7.
**Conclusion:** Smoking patients carrying the ecNOS4a polymorphism had a significantly increased risk to develop DN.

Crude Odds Ratios

| | **Any DN** OR (95% CI) | **Overt DN** OR (95% CI) |
|---|---|---|
| All patients; N cases = 122 | 1.65 | 2.09 |
| (48 overt); N controls = 200 | (1.01–2.68) | (1.08–4.02) |
| Never smoked; N cases = 38 | 0.85 | 0.90 |
| (11 overt); N controls = 61 | (0.34–2.12) | (0.21–3.77) |
| Smoked previously; N cases = 40 | 2.03 | 4.52 |
| (16 overt); N controls = 48 | (0.82–5.00) | (1.41–14.48) |
| Smoking now; N cases = 21 | 7.12 | 6.33 |
| (10 overt); N controls= 21 | (1.31–38.77) | (0.92–43.62) |

## 124

**Mannose-binding lectin is associated with development of micro-albuminuria in Type 1 diabetic patients - an inception cohort study**

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**Background and aims:** Inflammation and complement activation have been suggested to play a role in the pathogenesis of diabetic micro- and macrovascular complications. Therefore we evaluated the association between serum mannose-binding lectin (MBL) and development of persistent microalbuminuria in an inception cohort of 286 type 1 diabetic patients.
**Materials and methods:** All patients consecutively admitted to the Steno Diabetes Center between September 1st 1979 and August 31st 1984 with newly diagnosed type 1 diabetes were included in the inception cohort. MBL was measured with an immunoflourometric assay in 270 of the patients (159 men) after three years of diabetes duration, i.e. after initial glycaemic stabilization and prior to development of persistent microalbuminuria (>30 mg/24 hours in two out of three consecutive urine samples).
**Results:** During the median (range) follow-up of 18.0 (1.0–21.8) years, 75 patients progressed to persistent micro- or macroalbuminuria The median level (interquartile range) of MBL was significantly higher three years after onset of diabetes in patients later progressing to persistent micro- or macroalbuminuria: 2.232 (1.088-3.097)µg/ml vs. patients with persistent normoalbuminuria: 1.396 (0.492-2.731)µg/ml, $p=0.007$. The cumulative incidence (by life-table method) of persistent micro- or macroalbuminuria was 33% (95% CI: 27 - 40) in the cohort. In patients with serum MBL levels above the median (1.597 µg/ml), the cumulative incidence of persistent micro- or macroalbuminuria was 41% (31 - 50), as compared to 26% (17–34) in patients with MBL levels below the median MBL, log rank test: $p=0.003$. In a Cox proportional hazard model, serum MBL measured three

years after onset of diabetes was significantly associated with later development of persistent micro- or macroalbuminuria (hazard ratio: 1.17 (95% CI: 1.04-1.32) per 1 µg/ml increase in serum MBL, p=0.009). After adjustment for the confounding effects of gender, age, height of the patients, $HbA_{1c}$, systolic blood pressure, and smoking, MBL was independently associated with the development of micro- or macroalbuminuria (hazard ratio 1.26 (1.09 - 1.47) per 1 µg/ml increase in serum MBL, p=0.003).
**Conclusion:** High levels of MBL early in the course of type 1 diabetes is significantly associated with later development of persistent micro- or macroalbuminuria, suggesting that MBL may be involved in the pathogenesis of microvascular complications.

## 125

**Multilocus approach to the identification of genetic risk factors for diabetic nephropathy in Type 2 diabetes**

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**Background and aims:** Set association of selected genetic polymorphisms (40 single nucleotide polymorphisms (SNPs)) in 26 candidate genes on chromosomes 1, 3, 4, 6, 7, 12, 16, 17, 19, 20 and 22 with diabetic nephropathy (DN) was studied in patients with type 2 diabetes mellitus. Products of genes studied were components of renin-angiotensin system, other haemodynamic factors, antioxidant enzymes, cytokines and growth factors, AGE-receptors, extracellular matrix remodelation enzymes and others.
**Materials and methods:** A total of 650 unrelated Caucasian subjects were enrolled into study comprising three groups: diabetics with parallel DN (cases), diabetics without DN (controls 1) and non-diabetics (controls 2). DN diagnosis was based on assessment of albumin excretion rate (AER). Genotypes were detected by means of PCR-based methodology. Set association study was performed to find SNPs jointly associated with disease.
**Results:** An initial comparison of genotype frequencies in case and pooled control individuals furnished P-values below 0.05 for 3 SNPs located on the 6th chromosome, namely 2184A/G in the RAGE gene (Receptor of Advanced Glycation End products), 252A/G in the LTA gene (Lympho-Toxin-Alpha, formely TNFb) and A16V in the SOD2 gene (Mn superoxid-dismutase). Comparing each genotype with the other two combined, the strongest frequency differences between cases and controls were observed for genotype RAGE 2184GG and genotype LTA AA (P=0.03 each). Haplotype analysis furnished highly significant (P<0.0001) results. The highest odds ratio for cases versus controls was 3.58, which was observed for haplotype consisting of the following alleles: RAGE -429C/RAGE 2184G/LTA 252A/SOD2 16A.
**Conclusion:** The preliminary data indicate that certain polymorphisms in genes encoding AGE-receptors, antioxidant enzymes and cytokines could be regarded as contributors to genetic risk factors for DN in type 2 diabetes. Association of these polymorphisms with susceptibility to develop DN and rate of progression and severity of DN in type 1 diabetes is a subject of ongoing study.
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## 126

**Retinal vascular adaptations to blood pressure are impaired in Type 1 diabetes**

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**Objective:** Autoregulatory control of the retinal circulation is disturbed in diabetes (DM) but the incidence of diabetic retinopathy (DR) may be reduced by effective blood pressure (BP) control. Structural & functional changes in retinal arterioles also occur in hypertension and these may reflect microcirculatory adaptation. The aim of this study was to assess the influence of type I DM and BP on retinal geometry.
**Methods:** 44 normotensive (BP<140/90) twin pairs, aged 14–38 years were studied. At least one sibling had type I DM (and no DR) and the subjects were divided according to DM status. The two groups were similar in age, fasting lipids, BP and smoking habit. Standardised retinal photographs were acquired and digitised. Retinal arteriolar parameters measured were diameter (D), length-diameter ratio (L:D), tortuosity index (T) and bifurcation angle (ω).
**Results:** Data for normal (N) and DM subjects are shown in the table.
Normal subjects in the higher tertile for BP had narrower and more tortuous arterioles with increased L:D ratios. Older normal subjects had narrower bifurcation angles. These BP effects, consistent with previous work, were absent in DM subjects. In DM, increased arteriole diameters were associated with higher HbA1 c. Additionally, in a stepwise multivariate regression model, fasting glucose in DM subjects (p=0.005) and fasting triglycerides in normal subjects (p=0.002) showed independent correlation with tortuosity.
**Conclusions:** The usual pattern of adaptive geometric changes in retinal arteriolar structure associated with increasing BP is absent in DM. This failure of vascular adaptation may contribute to the progression of microvascular disease and retinopathy in diabetes.

| Geometry | Subj | Tertiles of | Lower T | Upper T |
|---|---|---|---|---|
| D (pixels) | N | BP | 18.7 ± 0.5 | 17.3 ± 0.3* |
| L:D | N | BP | 32 ± 2 | 41 ± 2* |
| T | N | BP | 1.05 ± 0.01 | 1.12 ± 0.03* |
| ω (degrees) | N | Age | 82 ± 2 | 76 ± 2* |
| D (pixels) | DM | HbA1 c | 17.8 ± 0.2 | 19.7 ± 0.3* |

Data expressed as means ± SEM. *p<0.05

# OP 22

# Cost and quality in diabetes care

## 127

**Cost-effectiveness analysis of improved blood pressure control of hypertensive patients with Type 2 diabetes mellitus**

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**Background and aims:** Hypertension in people with type 2 diabetes is associated with an increased risk of micro and macrovascular complications. The hypertension in diabetes studies so far reported, provide both the clinical information on micro and macrovascular complications, and the information on use of resources associated with treatment and managing complications, thereby allowing the cost-effectiveness of tight blood pressure control in patients with type 2 diabetes to be assessed. The present study was done to assess the cost-effectiveness of tight control of blood pressure in hypertensive diabetic patients, and to calculate the costing, cost analysis and assess the cost-effectiveness of the intended intervention. Incremental cost-effectiveness analysis and incremental cost per event-free year gained within the trial period of these patients was another objective.
**Materials and methods:** A total of 60 hypertensive patients with type 2 diabetes undergoing treatment at the Cardiology OPD, BIRDEM and NHN were selected purposively in this cross-sectional study and were interviewed in March 2004 with a preset questionnaire along with scrutinization of guide book records regarding the direct cost (cost of medical advice, investigations, medical and other treatment) and indirect cost [travel cost, cost of productivity loss and accompanying person(s)]. Of them 30 were hypertensive patients with type 2 diabetes having uncontrolled blood pressure & ill-managed (BP>120/80 mm/Hg) and 30 were hypertensive patients with type 2 diabetes having controlled blood pressure & well-managed (BP≤ 120/80 mm/Hg). A comparison was made between these two groups. The degree and extent of complications, treatment outcome, clinical effectiveness, functional level, consumer's out of pocket expense and indirect cost of consumers were calculated. Incremental cost-effectiveness analysis has been calculated for patients (mean age 52 years) with type 2 diabetes. The incremental cost per event-free year gained within the trial period was also calculated.
**Results:** Cost analysis in 60 patients showed that the total cost of treatment was US$ 26616.32 (direct cost US$ 17593.12 and indirect cost US$ 9023.2) with an average of US$ 443.60 per patient. On comparing the two groups, the cost of uncontrolled group was found to be higher by US$ 6657.74 than that of controlled group. The incremental cost of intensive management (well-managed group) was US$178 (US$95 to US$232) per patient and event-free time gained in the intensive group was 0.55 (0.18 to 0.92) years and the lifetime gain was 1.19 (0.79 to 1.81) years. The incremental cost per event-free year gained was US$356 (costs and effects discounted at 6% a year) and US$198 (costs discounted at 6% a year and effects not discounted).
**Conclusion:** Intensive blood pressure control in hypertensive patients with type 2 diabetes significantly increased treatment costs but substantially reduced the cost of complications and increased the event-free days. Timely management of patients with diabetic hypertension is both clinically beneficial and cost-effective. It can increase the interval without complications, and the cost-effectiveness ratio compares favorably with many accepted healthcare program. This indicates that comprehensive care can reduce the burden of cardiac events of diabetic patients even in a developing country.
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**Cost utility analysis of intensive blood-glucose control, tight blood pressure control and metformin in patients with Type 2 diabetes**

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**Background and aims:** While the United Kingdom Prospective Diabetes Study (UKPDS) has demonstrated that intensive blood glucose control and tight blood pressure control are cost-effective, the comparisons to date have been restricted to measuring outcomes in life years or end-point free time. Maximum comparability and usefulness for decision makers is obtained when outcomes can be expressed in common units, and the measure that has gained most currency amongst economists has been the quality adjusted life year (QALY). Therefore the aim of this study is to extend previous analyses by evaluating these UKPDS policies using QALYs as the outcome measure.
**Materials and methods:** Cost utility analysis based on patient level data from a randomized clinical controlled trial involving 4209 patients with newly diagnosed type 2 diabetes conducted in 23 hospital based clinics in England, Scotland and Northern Ireland as part of the UKPDS. Within trial data was supplemented with lifetime extrapolation using the UKPDS Outcomes Model, which is based on an integrated system of parametric equations using updated covariates. These equations were used to predict a natural history of the disease for each patients remaining lifetime. Three different policies were evaluated: intensive blood glucose control with sulphonylurea/insulin; tight blood pressure control of hypertensive patients; and intensive blood glucose control with metformin for overweight patients. Incremental cost-effectiveness ratios were calculated based on the net cost of health care resources associated with these policies and the estimated effectiveness in terms of the incremental QALYs gained from within trial effects. Costs and effects were discounted at the UK Treasury recommended rate of 3.5%.
**Results:** The incremental cost-effectiveness ratio (in year 2000 United Kingdom prices) for intensive blood-glucose control was £6,294 per QALY, and for blood pressure control was £703 per QALY. Metformin therapy reduced overall costs by £851 and increased quality adjusted life expectancy by 0.55 years.
**Conclusion:** Each of the three policies evaluated has a cost per QALY gained lower than that of many other accepted uses of health care resources, and therefore represent good value for money. The results provide an economic rationale for ensuring that care of patients with type 2 diabetes corresponds at least to the levels of these interventions.

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**Metabolic control in diabetes - the effect on health-related quality of life**

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**Background and aims:** Diabetes is a complex metabolic condition that requires diligent day to day management. Poor management and control of diabetes often leads to poor disease outcomes. The North West Adelaide Health Study is a cohort study that has been designed to allow cross sectional analyses at different points in time. This cross sectional study of quality of life in those with diabetes by HbA1 c level will allow deeper understanding of the effects of metabolic control of diabetes on health-related quality of life.
**Materials and methods:** A representative population sample of people aged 18 years and over living in the North Western region of Adelaide (n=4060) was recruited via telephone interviews to participate in the North West Adelaide Health Study. This study included a clinical assessment of participants' health. HbA1 c, or glycosylated haemoglobin, is a measure of the amount of glucose-bound haemoglobin and provides information on long-term glucose control. High HbA1 c was defined as those with a HbA1 c level of greater than 7%. A HbA1 c level of greater than 7% indicates poor glucose control over time, whereas a HbA1 c level of less than or equal to 7% indicates relatively good glucose control over time. Those with diabetes were defined as those who had a FPG level of at least 7.0 mmol/L, or those who self-reported being told by a doctor that they had diabetes.
Health-related quality of life was measured using the generic Short Form 36 (SF-36). The stages along the diabetes disease continuum were compared on their self-reported health status using the eight dimensions of the SF-36, namely physical functioning (PF), role-physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role-emotional (RE), and mental health (MH). Standardised scores were calculated for each SF-36 dimension by dividing the differences between the SF-36 scores for those with diabetes and the norm of the population without diabetes. The standard score for the study population without diabetes was set at zero. In interpreting the differences in standard scores between groups, an effect size of 0.4 was described as moderate, and an effect size of 0.6 was described as severe.
**Results:** The prevalence of diabetes was 6.5% (95% CI 5.8–7.3). The prevalence of high HbA1 c level among people with diabetes was 41.1% (95% CI 35.2–47.1). The prevalence of high HbA1 c among the total study population was 2.8% (95% CI 2.3–3.3).
Compared to those with diabetes with acceptable HbA1 c levels, the mean SF-36 scores of those with diabetes who had high HbA1 c levels were significantly lower on the Physical Functioning ($p<0.02$), General Health ($p<0.001$), and Vitality ($p<0.001$) subscales of the SF-36. A comparison of

standardised SF-36 scores by HbA1 c level among people with diabetes, and controlled for age and sex, showed that having diabetes and a HbA1 c level greater than 7% had a severe effect on Physical Functioning, Role Physical, and General Health, and a moderate effect on Vitality and Social Functioning. Compared to those without diabetes, having diabetes and a HbA1 c level less than or equal to 7% had a severe effect on Physical Functioning, and a moderate effect on Role Physical.
**Conclusion:** Poor glucose control among people with diabetes has a significant effect on health-related quality of life. Improving metabolic control of diabetes will not only slow the development of complications of the disease, but will also improve the health-related quality of life of people with diabetes.

## 130

**Socioeconomic determinants of the distribution of diabetes-attributable direct health care expenditure: The Fremantle Diabetes Study**
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**Background and aims:** The economic impact of diabetes is immense from a community and individual perspective. The aim of the present study was i) to examine how annual total direct "diabetes-attributable" health care costs (DAHCC) for type 2 diabetes were distributed in a community-based patient cohort, and ii) to determine whether socioeconomic variables were associated with cost distribution disparities.
**Materials and methods:** We studied 593 type 2 diabetic patients from a community-based cohort followed for a mean ± SD of 4.3 ± 0.4 years. DAHCC (total, prescription blood glucose lowering (BGL) and non-BGL medications, hospital, general practitioner (GP)) were calculated by applying a range of literature-derived attributable proportions for each diabetic complication for which the medication was likely to have been prescribed or for which the hospitalisation occurred as primary diagnosis. Results for the conservative low cost range are presented. Costs were societal (to both government and patient) calculated in year 2000 A$. Education level, English ability, household income and ethnic background were used as surrogates for socioeconomic status.
**Results:** Over the four-year period, total median annual DAHCC increased 14% from A$493 to A$564/patient ($P<0.001$), median annual BGL medication costs doubled from A$60 to A$120 ($P<0.001$) and diabetes-attributable non-BGL medication costs increased from A$15 to A$80/patient ($P<0.001$). Median annual/patient costs of GP visits were stable (A$69 at baseline and A$52 thereafter, $P=0.10$). Total hospital costs remained steady with an average of A$108,583/year for all participants ($P=0.57$). Cost distributions were skewed and hence square root-transformed before further analysis. Using multiple linear regression, and after adjusting for age, gender and a range of diabetes-related variables at study entry, both higher BGL medication and GP costs were significantly predicted by poorer English-speaking ability. Higher diabetes-attributable non-BGL costs were predicted by higher educational attainment, and higher hospital costs with non-Anglo-Celt, non-Mediterranean European ethnic background (former Yugoslavia, Eastern Europe, the Netherlands and Germany). Total costs were not predicted by any of the socioeconomic variables.
**Conclusion:** Consistent with our previous data showing increased insulin use amongst Southern Europeans, non-English speaking migrants have the greatest use of BGL therapy and may require correspondingly greater GP access. Patients with higher educational attainment had increased non-BGL costs, perhaps due to better knowledge of, and access to, health care. This may, however, be needs-based since Southern Europeans had lower education levels and better lipid profiles. Reasons for the inequitable use of hospital resources by ethnicity are unclear. There is, however, generally equitable distribution of resources for the treatment of diabetes and its complications in Australian urban communities.
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**Health-related quality of life in diabetes by stage of disease progression**
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**Background and aims:** Diabetes is recognised as a National Health Priority Area in Australia because of the significant burden that it places on the community in terms of health, social, economic and emotional costs. The North West Adelaide Health Study has been designed to segment a large representative population sample according to stage of disease to identify each segment's characteristics. The stages of diabetes progression are defined in this study as no diabetes, impaired fasting glucose (IFG), previously undiagnosed diabetes, and diagnosed diabetes. This cross-sectional study of quality of life by each stage along the diabetes disease continuum will allow deeper understanding of the effects of diabetes progression on health-related quality of life (HRQL).
**Materials and methods:** A representative population sample of people aged 18 years and over living in the North Western region of Adelaide (n=4060) was recruited via telephone interviews to participate in the North West Adelaide Health Study, which included a clinical assessment of their health. Participants were defined as having IFG if they had a fasting plasma glucose (FPG) level of at least 6.1 mmol/L and less than 7.0 mmol/L. People with diabetes were defined as those who had a FPG level of at least 7.0 mmol/L, or those who self-reported being told by a doctor that they had diabetes. Those with previously undiagnosed diabetes were defined as having a FPG level of at least 7.0 mmol/L but who did not report having been told by a doctor that they had diabetes.
HRQL was measured using the generic Short Form 36 (SF-36). The stages along the diabetes disease continuum were compared on their self-reported health status using the eight dimensions of the SF-36, namely physical functioning (PF), role-physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role-emotional (RE), and mental health (MH).
**Results:** The prevalence of IFG in this sample was 4.4% (95% CI 3.8 - 5.1). The prevalence of previously undiagnosed diabetes was found to be 1.0% (95% CI 0.7–1.4), and the prevalence of diagnosed diabetes was 5.5% (95% CI 4.8–6.2). The prevalence of all diabetes was 6.5% (95% CI 5.8–7.3).
There was a general trend for HRQL to be more impaired with progression along the diabetes continuum. This relationship remained even after controlling for the effects of age and sex. Those with IFG scored significantly lower than those with normal glucose levels on the PF ($p<0.01$) and GH ($p=0.03$) subscales of the SF-36. Those with undiagnosed diabetes scored significantly lower than those with normal glucose levels on the PF subscale ($p<0.001$) of the SF-36. Compared to those with normal glucose levels, those with diagnosed diabetes scored significantly lower on the PF ($p<0.001$), RP ($p<0.001$), GH ($p<0.001$), VT ($p<0.001$), SF ($p<0.001$), RE ($p<0.001$), and MH ($p<0.01$) subscales of the SF-36. Overall, those with diabetes (diagnosed and undiagnosed) scored significantly lower than those without diabetes on all subscales of the SF-36.
**Conclusion:** As position along the diabetes continuum approaches disease, HRQL decreases. This analysis of HRQL by stage of disease progression provides support for interventions that delay or halt the progression of diabetes.

## 132

**Quality of diabetes care as a predictor of the development of cardiovascular events: results of the QuED study**
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**Background and aims:** There is considerable pressure on health care systems to deliver high-quality care while controlling costs. Several public and private health care systems have adopted indicators based on combination of process and intermediate outcome measures but nobody had evaluated if this indicators could be associated to long-term outcome measures. The QuED Study has been designed to evaluate the relationship between quality of care delivered to individuals with type 2 diabetes and long-term outcomes. After 5 years of follow-up, we assessed whether a quality of care summary score estimated from baseline data, was able to predict the development of cardiovascular (CV) events.
**Materials and methods:** The summary score was calculated using process and intermediate outcome indicators relative to $HbA_{1c}$, blood pressure, LDL cholesterol, and microalbuminuria. The score was constructed considering the presence of at least one measurement in the past 12 months, the levels of control obtained, and the lack of a specific treatment despite the presence of inadequate control. The score ranged from 0 to 40, with a higher score indicating better quality of care. 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 score was an independent predictor of CV events incidence during five years. Analyses were adjusted for patient case-mix and physician-level clustering.

**Results:** Overall, 3235 patients with type 2 diabetes were enrolled, of whom 2448 were recruited by Diabetes Clinics and 785 by General Practitioners. During the follow-up, 492 patients (15.2%) developed a CV event. The CV events rate was strictly related to the quality of care score, being of 62.4% per 1000 person years in patients with a score ≤10, 54.8% per 1000 person years in those with a score between 15 and 20, and 39.8% per 1000 person years in those with a score >20. Multivariate analysis, adjusted for age, gender, duration of diabetes, smoking, history of CV event, presence and severity of diabetes complications and comorbidities, showed that the risk to develop a new CV event was 89% greater in patients with a score of ≤10 (RR=1.89; 95%CI 1.43-2.50) and 43% higher in those with a score between 10 and 20 (RR=1.43; 95%CI 1.14-1.79), as compared to those with a score >20. The mean quality score varied substantially across centers. Multilevel analysis documented that a difference between centers of 5 points in the mean quality score was associated with a difference of 16% in CV event risk (RR=0.84; 95%CI 0.72-0.98).
**Conclusion:** Our study documented for the first time a close relationship between quality of diabetes care and long-term outcomes. In the light of the great variability in the quality of diabetes care documented, it is reasonable to assume that a substantial proportion of CV events could be avoided by adopting more stringent therapeutic targets.

# OP 23

# Normal and abnormal islet cell growth and differentiation

## 133

**Sequential expression of elements of beta cell stimulus-secretion coupling follows islet hormone expression in human fetal life**

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**Background and aims:** Although cells expressing insulin can be detected early in human and rodent fetal life (8 weeks in humans), fetal islets show poor insulin secretory responses to glucose. Factors responsible for maturation of beta cell stimulus-secretion coupling in fetal life are undefined. Deficient secretory responses to glucose could result from delayed glucose stimulus-secretion coupling, for example by lack of glucose transporters (Glut-2), enzymes regulating metabolism of glucose (glucokinase) or ion channels transducing metabolic signals to increases in cytoplasmic calcium concentrations (K-ATP channels; KIR6.2 and SUR1). To investigate the time course of expression of proteins that participate in glucose sensing and insulin secretion in the pancreatic beta cell, we used immunohistochemistry and dual-labelling immunofluorescence on sections of human fetal and post-natal pancreas.
**Materials and methods:** Pancreases obtained with ethical approval from human fetuses (9-36 weeks gestation), from infants 6–8 months old and from adults were formalin fixed and embedded in paraffin. Consecutive sections were labelled with polyclonal or monoclonal antibodies to Glut-2, glucokinase, KIR6.2, SUR1, islet hormones and the duct marker cytokeratin-19. Co-expression of antigens was examined by dual and triple immunofluorescence and immunohistochemical labelling.
**Results:** Scattered insulin-positive cells and small cell clusters were observed at 9 weeks gestation, and from 16 weeks, beta cell clusters were associated with a 'cap' of endocrine (predominantly somatostatin and glucagon) non-beta cells, which was still present in the infant pancreas. Glut-2 labelling was detected in the 9 week pancreas, but largely within the ductal epithelium. From 16 weeks Glut-2 expression was detected in a proportion of insulin-positive cells; this proportion increased with gestational age. Glucokinase was confined to endocrine cells with clear labelling detected from 16 weeks. Components of the K-ATP channel (KIR6.2, SUR1) were initially detected prior to 16 weeks only in duct cells weakly positive for cytokeratin-19 and not in beta cells. From 18 weeks, these proteins were detected in increasing proportions of insulin-positive fetal pancreatic cells. All components were expressed in the beta cells of the adult pancreas.
**Conclusion:** The results demonstrate that elements of the stimulus-secretion coupling apparatus are expressed in only a small proportion of beta cells in early fetal development and that the K-ATP channel is expressed in ducts, rather than beta cells prior to 18 weeks. Deficiencies in development of stimulus-secretion coupling could therefore contribute to poor insulin secretion in early human fetal life.

## 134

**Endocrine regulation of beta cell mass through insulin receptor substrate signalling**

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**Background and aims:** The maintenance of an adequate β-cell mass is the result of a physiologic balance between β-cell proliferation and apoptosis. Several growth factors and their receptors have been implicated in β-cell growth.
The insulin signaling pathway plays an important role in β-cell function and differentiation. We have previously shown that double heterozygous knockouts of Insr/Irs1 and Insr/Irs2 and triple heterozygous Insr/Irs1/Irs2 respond with different β-cell compensation to insulin resistance. In the present study, we performed in vitro studies of islet proliferation to address the possibility that islet hyperplasia is caused by a circulating islet growth factor.
**Materials and methods:** We studied 2- to 16 week-old mice with combined heterozygous mutations of Insr/Irs1 and Insr/Irs2 and triple heterozygous

Insr/Irs1/Irs2. We measured iselt proliferation inβ TC3 cells and used hepatocytes as controls. We evaluated pancreas histology with respect to β-cell proliferation (Ki67 staining) and apoptosis (ApoTag peroxidase). Results were expressed as percentage of the total surveyed pancreatic area occupied by β-cells.
**Results:** In pancreas sections, we detected the largest islets in Insr/Irs1/Irs2 mice, while Insr/Irs2 mice showed a less marked increase in islets size. There were no differences in apoptosis rates at any age. Replication rates were elevated in Ir/Irs2 and Ir/Irs1/Irs2 mice, allowing us to conclude that increased β-cell proliferation, rather than increased apoptosis, is the underlying cause of the observed hyperplasia.
We next measured the ability of serum derived from the mutant mice to cause in vitro proliferation of β-cells. Serum from Insr/Irs2 mice caused a greater proliferation of cultured β-cells than serum derived from Insr/Irs1 mice. Stimulation of islet proliferation in response to serum from Insr/Irs2 mice was associated with increased MAP kinase activity and a 7- to 10-fold increase in MAPK phosphorylation. Sera from the double and triple heterozygous mice caused similar changes in patterns of protein tyrosine phosphorylation, with no difference in the activation of Insr, Irs2 and Grb2. Phosphotyrosine content of Irs3 was lower in cells incubated with Insr/Irs2 serum. We observed a two-fold increase in phospho-Akt content of cells exposed to Insr/Irs1 serum.
Addition of Insr/Irs2 serum to cultured β-cells caused tyrosine phosphorylation of a Mr190 kDa molecular species, distinct form Irs and the receptors for EGF and PDGF.
**Conclusion:** We conclude that islet hypertrophy in insulin resistance is partly determined by a response to circulating growth factors acting through the MAP kinase pathway to promote β-cell proliferation.

## 135

**c-Myc: double jeopardy in β-cell failure**
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**Background and aims:** Employing the ins-MycER$^{TAM}$ mouse model, which allows specific activation of c-Myc in β-cells, we previously reported that activation of c-Myc results in both loss of differentiation and apoptosis of adult β-cells leading to diabetes. Given the increasing interest in the role of c-Myc we now aimed to address outstanding questions:
i) Given the links between c-Myc activation and two major potential causes of β-cell failure, namely loss of differentiation and apoptosis, can preventing Myc-induced apoptosis prevent diabetes?
ii) Is β-cell apoptosis solely and directly a product of c-Myc activation, or does gluco-lipotoxicity arising under the influence of defective β-cell differentiation/function co-conspire with elevated c-Myc to induce apoptosis?
We therefore studied the effects of c-Myc activation on β-cell differentiation and glucose homeostasis in vivo when Myc-induced β-cell apoptosis was prevented by expression of Bcl-$x_L$. Following this we examined the effects of maintaining normoglycaemia during c-Myc activation on islet mass and beta cell apoptosis.
**Materials and methods:** Transgenic ins-MycER$^{TAM}$ mice and ins-MycER$^{TAM}$/Rip-Bcl-$x_L$ double transgenic mice were treated with 2 mg/day 4-hydroxy-tamoxifen (4OHT) with intra-peritoneal (IP) injections to activate c-Myc in β-cells. Pancreata were collected, fixed and embedded in media for histological examination and β-cell mass determination. Measurement of mean cross-sectional area was performed using IP Lab Spectrum software, by assessing insulin expression in sequential sections 100 μm apart.
**Results:** Hyperglycaemia was first apparent by day 3 (D3) of c-Myc activation and preceded any overt decrease in β-cell mass. Mice were examined after the following durations of c-Myc activation: D4, D5, D7, D10 and D14. The β cell cross-sectional area was reduced by 90% on D5 of ins-MycER activation (Student *t* test, $P<0.001$) and remained at these levels until the end of 4OHT treatment. Comparison with ins-MycER$^{TAM}$xBcl-$x_L$ double-transgenic mice, showed that Myc-induced apoptosis is prevented, and islet mass increases throughout under the influence of Myc-induced replication, but mice still develop early transient hyperglycaemia due to loss of β-cell differentiation (reduced insulin, isl-1, neurod1, pdx-1) and/or function, which is only reversed after substantial increases in β-cell numbers.
Treatment of ins-MycER$^{TAM}$ mice with 1 IU/day of s/c insulin glargine (Aventis), limited the extent of hyperglycaemia during c-Myc activation. After 5–7 days of c-Myc activation histological examination showed a reduced loss of β-cell mass in insulin treated animals compared to untreated animals, though in both cases β-cell mass was reduced compared to control or wild type islets without c-Myc activation.
**Conclusion:** c-Myc activation induces β-cell failure through a combination of loss of differentiation and apoptosis. Even in the absence of apoptosis, c-Myc activation results in diabetes through loss of differentiation. Intriguingly, metabolic disturbances arising through loss of β-cell differentiation may contribute in part to Myc-induced apoptosis.
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## 136

**The level of expression of the *Hnf1α* gene is critical for its function in pancreatic beta-cells in vivo**
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**Background and aims:** Human and mouse genetic studies have shown that Hnf1α (MODY3) is essential for normal β-cell function. However, it is not known if the requirement for Hnf1α is cell-autonomous, if it is restricted to specific developmental time-points, or if the expression levels are relevant to its function. We designed a mouse model intended to address these issues.
**Materials and methods:** We generated double transgenic mice capable of inducing Hnf1α expression with a tetracycline regulated activator driven by the insulin promoter. Appropriate crossings were made to produce double transgenic mice on *Hnf1α*$^{+/+}$, $^{+/-}$, or $^{-/-}$ backgrounds. Hnf1α expression was specifically induced in β cells during different developmental time points or postnatally.
**Results:** Induced double transgenic mice exhibited β-cell Hnf1α levels which greatly exceeded the low levels normally observed in wild type cells. Overexpression of Hnf1α during embryonic development caused β-cell apoptosis with activation of caspase-3, regardless of the *Hnf1α* locus genotype. Maintained overexpression of Hnf1α in β-cells up to 8 weeks of age resulted in severely decreased β-cell mass, abnormal islet architecture, and in consequence diabetes. Pre or postnatal reexpression of Hnf1α with this system was accordingly incapable of rescuing the diabetic phenotype of *Hnf1a*$^{-/-}$ mice. However, reexpression levels in double transgenics were heterogeneous, and those *Hnf1α*$^{-/-}$ β-cells in which Hnf1α was reexpressed at low levels exhibited partial restoration of the expression of the Hnf1α-dependent gene Glut2, despite the hyperglycaemic environment.
**Conclusion:** The results show that the concentration of Hnf1α, rather than solely its presence or absence, is critical for β-cells. Furthermore, they suggest that at least for some functions the role of Hnf1α in β-cells is cell-autonomous. We believe that these results have important implications for the development of gene therapy strategies for MODY3, and possibly for other β-cell transcription factor deficient states.

## 137

**Altered glucose homeostasis in transgenic mice expressing the human transient neonatal diabetes mellitus (TNDM) locus**
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**Background and aims:** Transient neonatal diabetes mellitus (TNDM) is a rare diabetic syndrome apparent in the first weeks of life, and again during early adulthood. At present, the relative contributions of reduced islet β-cell number and impaired β-cell function to the observed hypoinsulinaemia are unclear. The inheritance pattern of this imprinted disorder implicates over-expression of one or both genes within the *TNDM* locus: *ZAC*, which encodes a zinc finger protein that promotes apoptosis and cell cycle arrest, and *HYMAI*, which encodes an untranslated mRNA.
**Materials and methods:** To investigate the consequences for pancreatic development, we have developed a high-copy transgenic mouse line *TNDM29* carrying the entire human *TNDM* locus. The PAC used (dj340H11) was 195 kb and extended ~100 kb upstream of the *ZAC* and *HYMAI* transcription start sites and putative imprinting control region, and was thus expected to provide faithful expression and imprinting.
**Results:** *TNDM29* neonates and older adults displayed hyperglycemia and impaired glucose tolerance, and an increase in the proportion of animals displaying frank diabetes. The embryonic pancreas of *TNDM29* mice show reduced expression of endocrine differentiation factors (Ngn3, Pdx-1, Pax6) and a decreased number of insulin, glucagon and somatostatin-positive structures. By contrast, β-cell mass was normal or elevated at all postnatal stages, whilst pancreatic insulin content and peak serum insulin levels after glucose infusion were reduced in *TNDM29* neonates and adults. No evidence for insulin resistance was evident at any developmental stage by euglycaemic-hyperinsulinaemic clamp.

**Conclusion:** Expression of human *ZAC and HYMAI* in *TNDM29* transgenic mice recapitulates the key features of human TNDM, and implicates impaired development of the endocrine pancreas and β-cell function in disease pathogenesis. This transgenic mouse model therefore represents a useful tool for the further analysis of this interesting condition and may also shed light on changes in β-cell development and function in more common forms of type 2 diabetes mellitus.
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## 138

**VEGF expression in pancreatic islets is important for insulin delivery**
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**Background and aims:** Pancreatic islets have greater vascularity and blood flow than surrounding pancreatic exocrine tissue; the responsible molecular mechanisms are incompletely defined.
**Materials and methods:** By immunocytochemistry, we found that VEGF was expressed in all four islet cell types at a much greater level than in surrounding acinar cells. To test the hypothesis that VEGF expression is important for islet vascularization and function, we inactivated VEGF-A in a β cell-specific manner by breeding RIP-Cre and VEGF-A-loxP mice.
**Results:** While adult mice -/- for VEGF-A in β cells had normal pancreatic weight, insulin content, and islet architecture, -/- mice cleared glucose following intraperitoneal glucose at a considerably slower rate than +/+ mice. Plasma insulin levels, normalized for blood glucose after glucose administration, were significantly lower in -/- mice (117 ± 12 ng/g; n = 12) compared to +/+ mice (229 ± 17 ng/g; n = 12). Vascular density within islets was significantly reduced in -/- mice (760 ± 38 vessels/mm$^2$; n = 3) compared to wild type mice (1581 ± 95 vessels/mm$^2$ ; n = 3). Vascularization parameters such as area/vessel (83 ± 6 mm$^2$ in islets of -/- mice) and perimeter/vessel (46 ± 2 mm in islets of -/- mice) were also diminished in comparison to +/+ mice (120 ± 21 mm$^2$ ; 55 ± 4 mm), indicating a reduction in the number of capillaries per islet, vessel size, and/or branching. Isolated islets from -/- mice and +/+ mice had a similar insulin secretory pattern in a perifusion system indicating similar islet cell function. By electron microscopy, intra-islet endothelial cells in the pancreas from -/- mice had disordered endothelial cell ultrastructure and reduced fenestration.
**Conclusion:** Reduced islet expression of VEGF resulted in reduced islet vascularization, disordered endothelial cell structure, and a pre-diabetic phenotype similar to some defects in β cell gene expression. These data suggest that normal islet vascularization and insulin delivery into the vascular system requires islet expression of VEGF-A. These observations also have implications for the revascularization of transplanted islets.

# OP 24
# Lipid metabolism

## 139

**Non-sterified fatty acids (NEFA) release in *vivo* by visceral adipose tissue in humans**
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**Background and aims:** Obese humans with abdominal fat distribution are at increased risk for type 2 diabetes, dyslipidemia, and hypertension due to the presence of insulin resistance. In obese subjects high levels of NEFA are found, and potentially contribute to the insulin resistance state. One potential mechanism linking abdominal/visceral adiposity with insulin resistance is the liberation of non-sterified fatty acids (NEFA) from visceral depots to portal vein and them having direct effects on hepatic metabolism ("Portal Theory"). The aim of this study was to assess the NEFA release from visceral adipose tissue (VAT) in the fasting state among normal-weight and obese subjects.
**Materials and methods:** Sampling from gastro-epiploic veins and arteries from 16 insulin resistant severe obese subjects (BMI: 50.2 ± 10.4 kg/m$^2$) and 8 normal-weight subjects (BMI: 22.4 ± 3.4 kg/m$^2$) with normal glucose tolerance, were collected during elective abdominal surgical procedures. NEFA release from VAT were estimated by the arteriovenous differences across a omental fat depot due to measurements of NEFA levels in the gastro-epiploic veins and arteries. Brachial venous samples were also collected. NEFA were measured by enzimatic espectrofotometric methods.
**Results:** Obese subjects were insulin resistant when compared with lean subjects (Homa-IR= 11.3 ± 3.9 vs. 2.2 ± 0.6; p<0.0001). NEFA in the brachial vein were higher in the obese group (1.31 ± 0.43 mmol/L vs. 0.55 ± 0.11 mmol/L, p<0.001). No differences were found between peripheral vein and omental arterial samples in regard to NEFA levels. The arteriovenous gradiente of NEFA were virtually zero in both groups. Obese group: gastro-epiploic arterie = 1.30 ± 0.40 mmol/L, gastro-epiploic vein = 1.31 ± 0.41 mmol/L. Lean group: gastro-epiploic arterie = 0.54 ± 0.05 mmol/L, gastro-epiploic vein = 0.52 ± 0.08 mmol/L (p= NS for both groups).
**Conclusion:** These results confirm the presence of elevated NEFA levels and insulin resitance in severe obesity. Otherwise, there was no consistent difference in NEFA concentrations across visceral (omental) depot in the fasting state. Our findings imply that increased visceral fat should be predictive of, but not the source of, excess NEFA in human obesity. The "Portal Theory", which holds that increased release of NEFA from visceral adipose depots leads to insulin resistance through effects on the liver, lacks supporting evidence *in vivo*.
*Supported by: Fundação Roberto Rocha Brito*

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**Postprandial trafficking of dietary fat in skeletal muscle and liver *in vivo* in humans**
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**Background and aims:** Increased liver and muscle triglyceride levels are associated with insulin resistance. It is not known whether these fat stores are stable or turn over rapidly. We studied the postprandial dynamics of fat storage in these tissues and the appearance of dietary fat in plasma lipoproteins.
**Materials and methods:** Nine healthy volunteers (6M/3F; age 49.4 4.89 years; BMI 30.5 1.55 kg/m$^2$) were studied after a mixed meal containing 3 grams of $^{13}$C-labelled (99%) fatty acids. Storage of $^{13}$C triglyceride in liver and muscle (soleus) was measured using a novel $^{13}$C magnetic resonance spectroscopy protocol. Baseline tissue triglyceride levels were measured by $^{1}$H spectroscopy. The appearance of $^{13}$C was measured in plasma lipoproteins using isotope ratio mass spectrometry.
**Results:** Baseline liver triglyceride was 58.2 20.8 mmol/l and increased rapidly (peak increment 6.57 1.5 mmol/l at 6 hours; p = 0.003). At peak, 10% of meal triglyceride was stored as liver triglyceride. Baseline muscle triglyceride was 35.4 6.7 mmol/l and uptake peaked at 5 hours (0.46 0.5 mmol/l; p =NS). At peak, total body muscle triglyceride accumulation also represented 10% of meal triglyceride. $^{13}$C incorporation peaked at 6 hrs in the

plasma chylomicron-triglyceride fraction ($p < 0.01$) and at 8 hours in very low density lipoprotein-triglycerides ($p < 0.01$).
**Conclusion:** The dynamics of postprandial dietary fat trafficking in human liver and muscle has been quantified for the first time. Application of these methods to type 2 diabetes will illuminate the pathogenesis of increased triglyceride accumulation in insulin-sensitive tissues.

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**Fasting and postprandial fat oxidation is increased in patients with Type 2 diabetes mellitus**

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**Background and aims:** 41 years ago Randle suggested that glucose and fatty acids compete for oxidation in muscle. Abnormal fatty acid metabolism may impair glucose metabolism in patients with type 2 diabetes mellitus (DM). The extent to which fat oxidation is altered in DM remains unclear. We examined fat oxidation postabsorptively and following a mixed meal in subjects with and without DM.
**Materials and methods:** 45 DM and 45 control subjects were given a mixed meal labelled with 1,1,1-$^{13}$C-tripalmitin. Plasma and breath samples were collected postabsorptively and hourly for 6 hours postprandially. Triglyceride (TAG) and non-esterified fatty acid (NEFA) fractions were isolated from plasma and $^{13}$C enrichment in palmitic acid ($^{13}$C-PA) in each fraction determined using gas chromatography combustion IRMS. Area under the curve (AUC) was calculated using the trapezoidal method. Net fat oxidation was calculated using indirect calorimetry. Oxidation of $^{13}$C labelled dietary fat by peripheral tissues was calculated using the percentage excretion over time of administered $^{13}$C -tripalmitin as $^{13}CO_2$ in breath and whole body $CO_2$ excretion.
**Results:** DM subjects had higher fasting TAG (mean±SD 2.65 ± 1.82 vs 1.70 ± 1.28 mmol/l, p=0.006 ) and higher postprandial AUC TAG (20.6 ± 12.26 vs 13.56 ± 9.20 mmol/l/6 h, p=0.003) than control subjects. No significant difference was detected in postprandial AUC $^{13}$C-PA in the TAG fraction in DM compared to control subjects (66.45 ± 38.18 vs 54.16 ± 35.32ng/ml/6 h, p=0.118), which suggests similar chylomicron clearance in both groups. DM subjects had higher fasting NEFA (mean±SD 200.19 ± 113.67 vs 132.24 ± 45.38 mmol/l, p<0.0001), higher postprandial AUC NEFA (607.07 ± 205.43 vs 422.59 ± 140.92 mmol/l/6 h, p<0.0001) and higher postprandial AUC $^{13}$C-PA in the NEFA fraction (2.80 ± 1.38 vs 2.05 ± 0.84 ng/ml/6 h, p=0.003) than control subjects. Fasting fat oxidation was higher in DM subjects (2.81 ± 1.62 vs 2.00 ± 1.66g/h, p=0.009) and DM subjects showed a trend towards higher postprandial net fat oxidation (19.75 ± 9.39 vs 16.15 ± 8.22g/6 h, p=0.06). DM subjects had higher AUC breath $^{13}CO_2$ (9.81 ± 3.34 vs 7.98 ± 2.60%dose/6 h, p=0.003), which suggests increased oxidation of dietary fat in DM.
**Conclusion:** Patients with DM have increased fat oxidation in the fasting state and oxidise more dietary fat following a mixed meal than control subjects. This is likely to be due to an increased supply of NEFA to peripheral tissues. No difference between the groups was found in AUC $^{13}$C-PA in the TAG fraction which suggests that rates of chylomicron clearance are similar in DM and control subjects. Increased AUC $^{13}$C-PA in the NEFA fraction in DM subjects may therefore be due to reduced NEFA uptake into adipose tissue after chylomicron hydrolysis.
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**Stimulation of peroxisomal fatty acid oxidation and depletion of muscle triglycerides is associated with improvement of insulin sensitivity after metformin treatment**

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**Background and aims:** The available published information on the lipid lowering actions of metformin (MET) does not seem to match adequately with MET's effects on insulin sensitivity. To shed more light on the aforementioned, *in vivo* insulin action of rats with the high fat (HF) diet-induced insulin resistance (IR), treated or not with MET, was measured and evaluated in relation to circulating and tissue lipid levels, and tissue fatty acid (FA) oxidation.
**Materials and methods:** Male Wistar (Charles River, Prague) rats were fed for 3 weeks a standard experimental animal chow (PD) or the HF (70 cal %) diet. The HF fed rats were also given daily MET (250 mg/kg B.W.) by gavage. Insulin action was assessed by the euglycemic hyperinsulinemic (6.4 mU/kg B.W./min) clamp (EHC) in conscious, freely moving animals. Routine biochemical parameters in blood (glucose, insulin, FFA, triglycerides and glycerol) and tissue (liver and muscle) lipid content were determined using commercial kits. Gene expression of key enzymes of mitochondrial (CPT1 and CPT2) and peroxisomal FA oxidation (AOX) was measured in liver and skeletal muscle (m. gastrocnemius) by the RT PCR technique.
**Results:** Feeding rats the HF diet led to hypertriglyceridemia (C: 1.4 ± 0.1 vs. HF: 3.4 ± 0.4 mM) and elevated liver (C: 5.0 ± 0.4 vs. HF: 18.0 ± 1.2 µmol per g) and skeletal muscle (C: 1.7 ± 0.1 vs. 3.0 ± 0.04) TG content. MET treatment decreased muscle lipid content without any changes in blood and liver lipids. The HF diet-induced decrease of insulin stimulated glucose disposal (PD: 25.1 ± 0.5[a]; HF: 15.8 ± 0.4[b] mg/kg/min) during the EHC was significantly improved by the long-term metformin (HF+MET: 18.6 ± 0.3[c] mg/kg/min) treatment. Nevertheless, it was not able to normalize the GIR to the values of control animals. Measurements of gene expression for key enzymes of FA oxidation in skeletal muscle revealed that MET stimulates peroxisomal (AOX, HF: 1.4 ± 0.3 vs. HF+MET: 2.6 ± 0.4 arbitrary units, $p<0.05$), but not the mitochondrial pathway. No changes in FA oxidation were found in the liver.
**Conclusion:** In summary, the data indicates that 1) long-term administration of MET, given once daily, leads to an improvement of *in vivo* insulin action in the HF diet-induced IR; 2) it does not seem to be accompanied by a decrease of circulating lipid levels, although 3) an up-regulation of the β-oxidation pathway after MET treatment may prevent the muscle TG accumulation in the HF diet-induced IR. It is likely that the peroxisomal FA oxidation could be one of the principal targets for MET action.
*Supported by: Merck, Lyon, France and MVTS COST B17*

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**Effect of GLP-1 upon lipid metabolism in morbid obesity**

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**Background and aims:** GLP-1, apart from its insulin-like effect upon glucose metabolism in rat liver and rat and human skeletal muscle, exerts a dual action upon lipid metabolism in rat adipocytes, being lipogenic from $10^{-13}$ M and also lipolytic at higher concentrations; exendin-4 (Ex-4), structurally related to GLP-1, and its fragment 9-39 amide (Ex-9), both are lipogenic in rat adipocytes, and Ex-4, but not Ex-9, also lipolytic. In normal human adipocytes, GLP-1 also exerts a counteracting effect on lipid metabolism, its lipolytic action being perhaps mediated by cyclic AMP. In this work we have studied the effect of GLP-1, Ex-4 and Ex-9 upon lipid metabolism in adipocytes from morbidly obese patients.
**Materials and methods:** Adipocytes were isolated by enzymatic digestion from subcutaneous fat tissue obtained, previous informed consent given, from 14 morbidly obese female patients undergoing bariatric surgery (age: 42.2 ± 2.6 yr; BMI: 51.3 ± 1.5 kg/m$^2$; fasting plasma glucose: 107.2 ± 6.9 mg/dl; HDL: 43.5 ± 3.5 mg/dl). Lipolysis – glycerol release – and lipogenesis – $^{14}$C-Na acetate incorporation into lipids – were measured in $10^5$ cells incubated for 60 min. in the absence (control) and presence of ($10^{-13}$–$10^{-9}$ M) GLP-1, Ex-4 or Ex-9, and $10^{-9}$ M glucagon or insulin.
**Results:** In obese patients, compared to nine previously studied normal subjects, the glycerol release control value (21.2 ± 2.3 nmol/$10^5$ cells, n=14) was higher, although not significantly, and also higher ($p<0.05$) the increase exerted by $10^{-9}$ M GLP-1 (81 ± 7% Δ of control, n=14, $p<0.001$); the stimulatory effect of glucagon (87 ± 18% Δ, n=14, $p<0.001$) was apparently more pronounced than that in normal subjects; the same effect as that by $10^{-9}$ M GLP-1 was detected with $10^{-9}$ M Ex-4 (77 ± 6% Δ, n=7, $p<0.001$). Also compared to normals, the lipogenic control value (4.9 ± 0.3 nmol/$10^5$ cells, n=7) in obese patients was higher ($p<0.001$), and whereas the magnitude of the stimulatory action of insulin was maintained (46 ± 7% Δ of control, n=7, $p<0.001$), no effect could be detected by GLP-1 or Ex-4 at any concentration tested; yet, an increment in the control value, although moderate, was measured at $10^{-9}$ M Ex-9 (19 ± 7% Δ, n=7, $p<0.05$).
**Conclusion:** In adipocytes from morbidly obese patients, GLP-1 exerts a potent lipolytic effect, even higher than in normal subjects but, opposite to insulin, no lipogenic action is detected. These results may indicate an additional therapeutical benefit of GLP-1 in obesity.

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**Reduced fatty acids ameliorate leptin resistance in massive obesity. 24 hour profiles of fatty acids, leptin, cortisol and growth hormone**

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**Background and aims:** Leptin stimulates fatty acid oxidation and glucose uptake, thus preventing lipid accumulation in non adipose tissues. Gender, caloric intake and, mainly fat intake, hormones including cortisol and GH seem to regulate leptin secretion. Bilio-pancreatic diversion (BPD) is a bariatric surgical technique which produces lipid malabsorption, thus mimicking the effects of a very low fat diet.
**Aim** of the present study was to investigate the effects of lipid malabsorption on 24-h leptin, glucose and insulin, cortisol, and GH profiles in 8 severely obese women undergone BPD.
**Materials and methods:** 24-h blood samples for glucose and hormonal measurement at 60-min intervals were obtained before and 14 months after BPD. Fourier analysis and PULSEFIT computerized algorithm were applied to hormonal profiles to identify diurnal variability and pulses. Insulin sensitivity was estimated by euglycemic hyperinsulinemic glucose clamp; body composition by dilution method, energy expenditure (EE) was measured in the calorimetric chamber and intake was computed by 7-days diary recall method. Muscle biopsies were obtained to quantify acetyl-coenzyme A carboxylase 2 (ACC2) $_{m}$RNA.
**Results:** After BPD, we found statistically significant changes in 24-h EE (from 11300 ± 1457 kJ to 7278 ± 773 kJ; P<0.0001); whole body glucose uptake (from 0.274 ± 0.022 to 0.573 ± 0.027 mmol/$kg_{FFM}$/min; P<0.0001); skeletal muscle ACC2 $_{m}$RNA (from 452.82 ± 76.35 to 182.45 ± 40.69% of cyclophilin mRNA after BPD; P<0.0001). After BPD, leptin AUC decreased from 1004.94 132.95 ng·h·$ml^{-1}$ to 286.10 59.86 ng·h·$ml^{-1}$ (P=0.002), as well as the maximum diurnal variation, or acrophase, (from 22.60 ± 2.79 ng/ml, to10.27 ± 1.70 ng/ml; P=0.001). The pulsatility index (PI) increased (P=0.02) from 1.050 ± 0.004 ng·ml·$min^{-1}$ to 1.084 ± 0.005 ng·ml·$min^{-1}$.The leptin plasma clearance (CR) increased from 0.0010 ± 0.0001 to 0.0020 ± 0.0001 $min^{-1}$ (P=0.01). The average number of peaks were unchanged. Insulin AUC (from 4908 ±786 to 1364± 118 pmol · h · $l^{-1}$; P=0.005) and insulin acrophase (475.86 ± 61.64 pM vs. 172.14 ± 12.39 pM; P = 0.001) were significantly reduced after BPD; insulin PI increased (1.53 ± 0.13 vs. 1.10 ± 0.12 pM/min, P=0.01) similarly to the insulin CR (from 0.005 ± 6·$10^{-4}$$min^{-1}$ to 0.009 ± 4·$10^{-4}$ $min^{-1}$; P=0.01). GH AUC and GH acrophase respectively increased from 11.17 ± 2.58 to 37.25 ± 1.41 μg · h · $l^{-1}$ (P<0.0001) and from 0.91 ± 0.20 μg · l·$min^{-1}$ vs 4.58 ± 0.80 μg·l · $min^{-1}$ (P=0.0001). The GH PI increased (1.20 ± 0.04 μg · l · $min^{-1}$ vs 1.70 ± 0.13 μg · l · $min^{-1}$; P=0.024), as well as the GH CR (0.006 ± 0.001 vs 0.010 ± 0.002 $min^{-1}$; P=0.025). No significant changes were observed in the cortisol AUC, acrophase, PI and CR. In a stepwise regression including insulin, GH and glucose levels ($R^2$= 0.87), changes in Glucose/Insulin (P=0.029) and changes in FFAs (P=0.002) were the most powerful predictors of leptin variations.
**Conclusion:** Our data suggest that: 1) the leptin resistance observed in obese subjects might be due to increased storage of fatty acids as intramuscle triglycerides; 2) the reduction of both leptin secretion and ACC2 $_{m}$RNA after BPD might be related to decreased lipogenesis and increased skeletal muscle oxidation of FFAs; 3) the reduced levels of circulating leptin might stimulate GH secretion.

# OP 25
# Insulin therapy in Type 2 diabetes

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**Effects of insulin vs. glibenclamide in recently diagnosed Type 2 diabetic patients: a 4-year follow-up**

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**Background and aims:** In the ongoing IVS (Insulin vs. Sulphonylurea) study we have investigated the effects of early insulin vs. glibenclamide treatment in recently diagnosed Type 2 diabetic patients. Recently we reported data from 0–2 year of the study. We now present results after four years of treatment.
**Materials and methods:** 34 patients with ICA-negative Type 2 diabetes diagnosed 0–2 years before inclusion entered this Swedish multicenter trial and were randomized to either 2 daily injections of pre-mixed 30% soluble and 70% NPH insulin or glibenclamide (3.5-10.5 mg daily). C-peptide-glucagon tests were performed yearly after 2 as well as after 3 days of temporary withdrawal of treatment.
**Results:** The C-peptide response increased significantly after 1 and 2 years in the insulin-treated group, whereas it was decreased in the glibenclamide-group (p=0.01 and p=0.02, respectively, for difference between groups). Fasting proinsulin levels had increased in insulin-treated group relative to glibenclamide-treated group (p<0.005) after 4 years. Fasting insulin levels tended to increase in insulin- vs. glibenclamide-treated patients (p<0.07) after 2 years. When including the final in-study HbA1 c for 4 glibenclamide-treated patients, who were excluded earlier because of treatment failure, end-point HbA1 c levels were lower in the insulin- vs. glibenclamide-treated group (p<0.05). The daily dose of insulin increased slightly, but significantly, during the study from 20.1 ± 7.7 U at year 1 to 24.9 ± 11.0 U at year 4 (p<0.01). Glibenclamide increased from 2.8 ± 2.0 mg daily at year 1 to 4.5 ± 3.4 mg daily at year 4 (p<0.05). Weight increased more in insulin-treated group (+4.4 ± 0.8 kg) than in glibenclamide-treated (+0.3 ± 1.0 kg) (p<0.005 between groups), but did not increase year 3-4.
**Conclusion:** In a four year perspective insulin treatment in recently diagnosed Type 2 diabetic patients had favourable effects on beta-cell function, and ameliorated metabolic control compared to glibenclamide-treated patients. Insulin-treated patients increased weight more than glibenclamide-treated patients, but the increase was modest and attenuated with time. Early insulin treatment is a viable therapeutic treatment in Type 2 diabetic patients.

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**Initiation of insulin glargine in Type 2 patients with suboptimal glycaemic control on twice-daily premix insulin: results from the AT.LANTUS trial**

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**Background and aims:** Maintenance of good glycaemic control slows development of diabetic complications. Premix insulin regimens are commonly used for Type 2 diabetes patients to improve metabolic control, either alone (≥2 injections/day) or with oral antidiabetic agents (OADs). The AT.LANTUS trial compared insulin glargine (LANTUS®) initiation and maintenance using one of two treatment algorithms (Algs) in Type 2 subjects poorly controlled on their previous regimen. Comparisons were made based on severe hypoglycaemia, glycaemic control and insulin dose. This subanalysis study established whether glycaemic control could be improved in patients switching from premix insulin (+/-OADs) to a once-daily insulin glargine-based regimen.
**Materials and methods:** This was a 24-week, multinational (59 countries), multicenter (611 centers), randomized (4961 patients), open study comparing two Algs. Alg 1 was a visit-based titration using 2–8 IU increments (10 IU initiation dose for insulin-naïve). Alg 2 involved patient self-titration

of 2 IU every 3 days (first dose was based on fasting blood glucose [FBG] for insulin-naïve). The titration was based on target FBG ≤5.5 mmol/L. Severe hypoglycaemia was defined as patient requiring assistance and blood glucose <2.8 mmol/L. This abstract reports change in glycaemic control for patients who changed from twice-daily premix insulin to once-daily insulin glargine alone or with prandial insulin and/or OADs.
**Results:** Significant improvement was observed in $HbA_{1c}$ in patients previously on twice-daily premix insulin +/-OADs following the switch to a once-daily insulin glargine-based regimen (Table). All patients showed a significant baseline to endpoint decrease in $HbA_{1c}$ ($p < 0.001$), achieved with a very low incidence of severe hypoglycaemia (≤2.2% for any insulin glargine treatment regimen) in a sample of patients at an advanced stage in their disease (full population >12 yr disease duration; >5 yr prior insulin therapy).
**Conclusion:** The analysis of this sub-population of patients previously receiving premix insulin shows that optimization of basal insulin therapy with once-daily insulin glargine is safe, easy to initiate and results in significant improvements in glycaemic control (>1.4% $HbA_{1c}$ decrease). In combination with prandial therapies (prandial insulin and/or OADs), insulin glargine offers additional glycaemic benefits. Thus, this study shows that insulin glargine is useful in facilitating titration regimens with normal glycaemic targets in diverse care settings.

| $HbA_{1c}$ (%) | | Agorithm 1 baseline | Algorithm 1 endpoint | Algorithm 2 baseline | Algorithm 2 endpoint | Overall baseline-endpoint Δ (Incidence severe hypoglycaemia %) |
|---|---|---|---|---|---|---|
| **Prior BD premix insulin** | Insulin glargine alone (n=169) | 8.9 ± 1.3 | 8.2 ± 1.5 | 8.8 ± 1.3 | 8.3 ± 1.3 | *-0.7 ± 1.6 (1.2%) |
| | Insulin glargine + BD prandial (n=89) | 9.2 ± 1.3 | 7.6 ± 1.3 | 9.3 ± 1.1 | 8.0 ± 1.1 | *-1.4 ± 1.4 (2.2%) |
| | Insulin glargine + >BD prandial† (n=83) | 9.0 ± 1.1 | 7.8 ± 1.1 | 9.3 ± 1.2 | 7.7 ± 1.2 | *-1.4 ± 1.3 (0%) |
| | Insulin glargine + OD prandial† (n=15) | 8.1 ± 1.0 | 7.9 ± 1.2 | 9.4 ± 1.6 | 7.9 ± 1.0 | -0.8 ± 2.0 (0%) |
| **Prior BD premix insulin + OADs** | Insulin glargine + OAD (n=311) | 8.9 ± 1.3 | 8.3 ± 1.4 | 8.7 ± 1.1 | 8.0 ± 1.2 | *-0.7 ± 1.4 (<1%) |
| | Insulin glargine + BD prandial† +OAD (n=74) | 9.0 ± 1.3 | 7.7 ± 1.0 | 8.9 ± 1.3 | 7.7 ± 1.4 | *-1.3 ± 1.3 (0%) |
| | Insulin glargine + > BD prandial† + OAD (n=78) | 9.3 ± 1.1 | 7.8 ± 1.1 | 9.0 ± 1.2 | 7.4 ± 0.9 | *-1.5 ± 1.2 (0%) |
| | Insulin glargine + >BD short-acting insulin + OAD (n=43) | 9.2 ± 1.0 | 7.8 ± 0.8 | 8.8 ± 1.0 | 7.5 ± 0.8 | *-1.3 ± 1.1 (0%) |
| | Insulin glargine + OD prandial† + OAD (n=26) | 8.8 ± 1.1 | 7.7 ± 0.9 | 9.2 ± 1.4 | 7.3 ± 0.6 | *-1.4 ± 1.4 (0%) |

* $p < 0.001$; BD=twice-daily; OD=once-daily; †Prandial includes regular and short acting, unless otherwise stated

*This study was supported by Aventis Pharma.*

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**Triple therapy in Type 2 diabetes: benefits of insulin glargine over rosiglitazone added to combination therapy of sulfonylurea plus metformin in insulin-naïve patients**

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**Background and aims:** Combination sulfonylurea plus metformin (SU+MET) remains the most common regimen for managing Type 2 diabetes; however, sustained glycemic control often requires triple therapy. To assess the relative merits of adding basal insulin or a thiazolidinedione (TZD) as the third agent, we compared the efficacy and safety of add-on insulin glargine (LANTUS®) versus rosiglitazone (ROS) in insulin-naïve patients on SU+MET with Type 2 diabetes.
**Materials and methods:** In this 24-week, multicentre, randomized, open-label, parallel trial, 217 patients ($HbA_{1c}$ 7.5–11%, body mass index [BMI] >25 kg/m²) on ≥50% SU+MET received add-on insulin glargine 10 units/day or ROS 4 mg/day. Insulin glargine forced titration to target fasting plasma glucose (FPG) ≤5.5 mmol/L (≤100 mg/dL) was lower than in the Treat-To-Target study. Increases in ROS dose to 8 mg/day were permitted any time after 6 weeks following treatment initiation if FPG >5.5 mmol/L (>100 mg/dL).
**Results:** Mean age, diabetes duration, BMI and baseline $HbA_{1c}$ (8.8–8.7%) were similar in both groups. Insulin glargine yielded better FPG values compared with ROS (-3.6 ± 0.22 mmol/L [-65 ± 4 mg/dL] vs -2.6 ± 0.22 mmol/L [-46 ± 4 mg/dL]; p=0.001). Change in $HbA_{1c}$ from baseline was similar between both groups (-1.7 ± 0.1% insulin glargine-treated patients vs -1.5 ± 0.1% ROS-treated patients), but reductions were significantly greater with insulin glargine when baseline $HbA_{1c}$ was ≥9.5%. The final dose/day was 38 ± 26 IU for insulin glargine versus 7.1 ±2 mg for ROS. Overall, hypoglycemia was similar between the two treatment groups. However, more insulin glargine-treated patients experienced confirmed symptomatic nocturnal hypoglycemia at <3.9 mmol/L (<70 mg/dL) but not at <2.8 mmol/L (<50 mg/dL). Patients treated with ROS experienced numerically more severe events than those treated with insulin glargine. Insulin glargine treatment was associated with significantly improved total cholesterol, low density lipoproteins (LDL) and triglyceride levels (10.9 to 10.4 mmol/L [196 to 187 mg/L], 6.5 to 6.4 mmol/L [117 to 115 mg/L] and 12.1 to 9.8 mmol/L [217 to 176 mg/L], respectively); ROS was associated with raised total and LDL cholesterol 10% and 13%, respectively (p=0.0001). Insulin glargine-treated patients had significantly less weight gain than ROS-treated patients (1.6 ± 0.4 kg vs 3.0 ± 0.4 kg; p=0.02), experienced fewer adverse reactions (6 vs 28%; p=0.0001) and no peripheral edema (0 vs 12.5%). It was calculated that treatment with insulin glargine saved US$397/patient over the 24-week period compared with ROS.
**Conclusion:** In patients with Type 2 diabetes receiving SU+MET, the addition of low-dose insulin glargine resulted in better FPG levels, a greater reduction in $HbA_{1c}$ when baseline was ≥9.5% and basically similar hypoglycemic profiles than the addition of ROS. Unlike ROS, insulin glargine was associated with fewer adverse reactions, no edema, less weight gain and salutary lipid changes at a lower cost of therapy. Conceivably, at higher doses, insulin glargine has the potential for greater glycemic benefits.
*This study was supported by Aventis Pharma.*

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**Multiple injection therapy (MIT) displaces conventional insulin therapy (CIT) as standard therapy in patients with Type 2 diabetes though not producing superior results after intervention by a structured treatment and teaching program in member hospitals of the AKD (working group for clinical diabetology)**

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**Background and aims:** Multiple injection therapy (MIT), a form of intensified insulin therapy with preprandial normal insulin and, if necessary intermediate or long acting insulin overnight, became standard therapy in patients with type-2-diabetes and displaced conventional insulin therapy (CIT) with premixed normal and long acting insulin twice a day though superior results for glycemic control, weight gain or improved quality of life

has not been convincingly shown by a randomized controlled study. All patients with type-2-diabetes taking part in the assessment of treatment quality in the AKD from years 2001 to 2004 were evaluated.
**Material and methods:** Personally conducted patient examination (Hba1 c, Blood pressure, weight) of a representative sample of at least 60 patients per ASD hospital 12 to 15 months after participating in a structured treatment and teaching program. Hba1 c is given as relative value (original Hb1ac/ normal mean of the local method). Patients with 1 or 2 insulin injections/d were regarded to receive CIT, with more than 2 MIT. Data of 56 member hospitals were analysed (total number 4856, n=3185 could be analysed respectively, age 62,0 y., Diabetes duration 10,9 y.).
**Results (CIT, n=719/MIT, n=2466, p-value):** The share of CIT shrank from 21% in 2002 to 15% in 2004. Before intervention patients receiving CIT were older (66,3/60,7y.; <0,0001) had lower BMI (29,2/30,4 kg/m$^2$; <0,0001), insulin need (30,6/44,0 IE/d; <0,0001), took more oral antidiabetic drugs (OAD) (46/39%; 0,003) and controlled blood sugar less often (7,2/14,6 per week, <0,0001). Diabetes duration (10,8/10,9), relative Hba1 c (1,69/1,69), blood pressure (systolic 144,3/141,6, diastolic 81,2/81,9) did not differ significantly. At reevaluation after 1 year both patient groups had significantly improved Hba1 c (<0,0001) and increased BMI (<0,0001). Diastolic (81,1/80,4, 0,150, 81,9/81,4, 0,002) but not systolic (143,9/143,1, 0,35, 141,6/141,6, 1,0) blood pressure decreased significantly. At reevaluation Hba1 c (1,44/1,41, 0,001) did differ significantly but marginally, BMI increase (+0,36/+0,54 kg/m$^2$) was more pronounced in the MIT-group. MIT-patients significantly performed more blood sugar self controls (15,7/22,3 per week; 0,0001), needed more insulin (40,0/55,5 IE/d; 0,0001; 0,48/0,63 IE/kg body weight; 0,0001) and made more insulin injections (1,9/4,1 inj./d; 0,0001). OAD use was similar in both groups (26/24%; 0,42).
**Conclusion:** One year after applying a structured teaching program in patients with type 2 diabetes metabolic control is only marginally better in MIT compared to CIT treated patients in spite of a more demanding therapy (more injections/d, higher frequency of blood sugar self controls/d). Since clear indications which patient may profit most, or suffer least, from which form of therapy are missing and proofs of superiority of MIT regarding long term benefits are still lacking CIT should not be neglected as viable, good alternative in insulin therapy.

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**Improved glycaemic control in severely insulin resistant, insulin-treated diabetic patients with U500 Human Actrapid over two year follow-up**
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**Introduction:** Some patients with Type 2 Diabetes (T2DM) are profoundly insulin resistant and require large insulin doses when insulin treated. However, such large volumes of subcutaneous conventional U100 insulin can be uncomfortable, and many of these patients have poor glycaemic control. One therapeutic option is U500 Human Actrapid, thus reducing the insulin volume by 80%.
**Methods:** We undertook a retrospective audit of patients requiring U500 Human Actrapid on account of poor glycaemic control and large insulin doses. The effects of U500 Human Actrapid on HbA1 c, weight, lipids and blood pressure were assessed over a two year follow-up period.
**Results:** Sixteen patients (13 having T2DM, two labelled as having Type 1 Diabetes and one with secondary diabetes due to haemochromatomasis) were followed for 23 ± 11.45 months (range 3-36 months). Five were Indo-Asian (3 male, 2 female) and 11 were white European (2 male, 9 female). Six patients were receiving Metformin, and four were receiving a thiazolidinedione (two each on Rosiglitazone and Pioglitazone). Mean insulin dose decreased from 313.8 units/day (2.78 unit/kg) to 261 units/day (2.18 units/kg) (ns). However, mean HbA1 c improved from 11.34 ± 3.27% to 8.97 ± 1.89% ($p$ =0.01), a mean decrease of 2.37% over the two years. No significant change was seen in systolic blood pressure (136.5 ± 16.9 Vs 140.4 ± 16.4 mmHg), and diastolic blood pressure (76.6 ± 11.2 Vs 76.5 ± 12.1 mmHg). Improved glycaemic control was associated with a trend to gain weight (112.7 ± 31.0 kg Vs 119.2 ± 33.1 kg, *p=0.55*). There were no differences in lipid parameters: total cholesterol (4.93 ± 0.81 mmol/l Vs 4.96 ± 0.65 mmol/l) and triglycerides (3.46 ± 1.70 mmol/l Vs 3.26 ± 1.77 mmol/l). U500 Human Actrapid was discontinued in six patients (two switched to insulin pump therapy, three on account of improved glycaemic control and lower insulin requirements, and one after bariatric surgery), but subsequently required reinitiating in two patients.
**Conclusion:** This audit demonstrates that poor glycaemic control in severely insulin resistant, insulin-treated diabetic patients can be substantially improved over a two year period with U500 Human Actrapid.

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**Treatment with insulin detemir provides improved glycaemic control and less weight gain compared to NPH insulin in people with diabetes**
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**Background and aims:** Near-normal glucose control has been shown to delay or prevent the development of diabetic late complications. The efficacy and safety of the new basal soluble insulin analogue, insulin detemir was recently compared with NPH insulin (NPH) in a number of clinical phase III trials. To investigate the overall effects of insulin detemir relative to NPH insulin, we conducted a meta-analysis comparing glycaemic control and development in body weight between insulin detemir and NPH insulin.
**Materials and methods:** The analysis included 6 multinational, open-label, randomised phase III trials in people with Type 1 diabetes (insulin detemir: n=1336, NPH: n=814) and Type 2 diabetes (insulin detemir: n=536, NPH: n=363), treated between 16 and 24 weeks, on a basal-bolus regimen with insulin detemir or NPH in combination with pre-meal regular insulin or insulin aspart.
**Results:** Subject characteristics were well balanced between treatment groups. $HbA_{1c}$, fasting plasma glucose (FPG) and weight at the end of treatment were analysed by an ANOVA with treatment and country as fixed effects and covariate adjustment for baseline values. Variation in home-measured fasting blood glucose (FBG) was analysed using a likelihood ratio test.

| | Insulin Detemir | | NPH Insulin | | Difference Detemir – NPH | |
|---|---|---|---|---|---|---|
| | N | Mean (SE) | N | Mean (SE) | Mean | 95% CI |
| HbA1 c (%) | 1763 | 7.79 (0.03) | 1114 | 7.88 (0.03) | -0.09 | (-0.15 ; -0.03) |
| FPG(lab) (mmol/L) | 1275 | 9.70 (0.15) | 626 | 10.80 (0.18) | -1.10 | (-1.46 ; -0.73) |
| Weight change (kg) | 1759 | 0.01 (0.09) | 1108 | 0.75 (0.10) | -0.74 | (-0.95 ; -0.53) |
| | N | SD (CV) | N | SD (CV) | p-value | |
| Within-person variation FBG (mmol/L) | 1747 | 2.55 (33.3) | 1102 | 3.06 (37.5) | <0.0001 | |

The analysis of FPG(lab) only included 4 of the 6 trials. CI: confidence intervals. CV: coefficient of variation
**Conclusion:** Treatment with insulin detemir provides improved glycaemic control, as measured by $HbA_{1c}$ and less variable fasting blood glucose, and results in a more stable body weight compared to NPH insulin in people with diabetes.
*The Study was sponsored by Novo Nordisk*

# OP 26

# Insulin resistance in Type 2 diabetes

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**Long-term metabolic effects of rosiglitazone on renal function in poorly controlled Type 2 diabetics**

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**Background and aims:** The goal was to assess the 1-year efficacy and safety of the addition of rosiglitazone instead of metformin to existing sulfonylurea therapy in inadequately controlled type 2 diabetic patients with mild renal impairment.
**Materials and methods:** In this randomized, single-blind, parallel-group study, patients were randomized to receive either rosiglitazone 4 mg plus existing sulfonylurea therapy without metformin (n=48) or only existing sulfonylurea including metformin therapy (n=44) for a period of 1-year.No significant difference were exist by means of age, diabetes duration, HbA1 c, body mass index, mean blood pressure, mean macroalbuminuria, serum creatinine, lipid and electrolyte levels between the groups. All patients were macroalbuminuric (UAE rate >300 mg/24 hr in a consecutive 3 measurements) with mild renal failure (mean serum creatinine 152.4 ± 24.2 µmol/L). All patients had hypertension treated with angiotensin-converting enzyme inhibitor in combination with an average of 3 other antihypertensive drugs. There were no changes in the medications used either for control of blood pressure or diabetes during the study period.
**Results:** The mean changes in HbA1 c were -1.3% with rosiglitazone (baseline 8.22%) and -0.4% with existing therapy (baseline 8.01%) (treatment difference p<0.01; 95% confidence interval (CI) -1.21, -0.8). Rosiglitazone addition to existing sulfonylurea therapy significantly reduced triglycerides (-14 vs. -3%; p<0.006) and increased HDL cholesterol (12 vs. 7%; p<0.01) compared with existing sulfonylurea therapy with metformin. LDL cholesterol was increased 4% by the addition of rosiglitazone and decreased 6% by the existing therapy (p<0.001). Urinary albumin-to-creatinine ratio was reduced by 27% in the rosiglitazone group and increased 4% in the existing sulfonylurea group (95% CI 0.97-1.27; p=0.004). Significant declines in systolic blood pressure -5.3 ± 0.4 mmHg and diastolic blood pressure -2.3 ± 0.2 mmHg (p<0.001, p<0.05) and urinary albumin excreation rate -97 ± 23 mg/24 h were achieved with addition of rosiglitazone.
**Conclusion:** Rosiglitazone was effective and well tolerated that may provide additional beneficial effects when added to sulfonylurea therapy in this population of patients with mild renal impairment.

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**Effect of pioglitazone on beta-cell function in metabolic syndrome patients with impaired glucose tolerance**

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**Background and aims:** Pioglitazone is a insulin-sensitizing agent from the thiazolidinedione group of compounds that has been shown to be effective in improving insulin sensitivity in persons with insulin resistance (IR). However, the effect of pioglitazone administration on insulin secretion in impaired glucose tolerance (IGT) patients has not been fully evaluated. The present study was thus undertaken to determine potential effect of pioglitazone on beta-cell function in patients with metabolic syndrome (MS) who have IGT.
**Materials and methods:** 22 MS patients (13 woman and 9 man, age: 52 ± 10 yr, body mass index: 26.3 ± 2.5 Kg/m$^2$) with IGT were treated with pioglitazone for 4 months. Samples for fasting plasma glucose, immunoreactive insulin (IRI) and proinsulin (PI) were obtained before and after therapy. Each subject underwent an intravenous glucose tolerance test (IVGTT) and additional hyperglycemic (11.1 mM) clamp study was performed in 11 subjects respectively at baseline and after treatment. The acute insulin response (AIR) was computed as the incremental area under the insulin curve from 1-10 min during IVGTT. Glucose disappearance rates (coefficients *K*) were calculated from the slope of the logarithm of the plasma glucose concentration between 1 and 30 min. The second-phase insulin response (2ndIR) was expressed as the average plasma insulin concentration during the last hour of the hyperglycemic clamp and insulin sensitivity index (ISI) was calculated by dividing the average glucose infusion rate during the last 60 min of the clamp by the average plasma insulin level.
**Results:** Pioglitazone treatment resulted in significant decreases in fasting plasma glucose and IRI concentrations [5.84 ± 0.65 mM *vs.* 5.05 ± 0.44 mM (P < 0.01) and 17.66 ± 6.58 µU/ml *vs.* 11.89 ± 2.58 µU/ml (P<0.01), respectively]. And a significant decrease in PI level was also observed (15.75 ± 12.03 pmol/L *vs.*7.48 ± 5.87pmol/L, P < 0.001.The distribution of values was skewed, so the statistics was based on natural logarithm). Although AIR was unchanged (500.2 ± 308.5 µU min/ml *vs.*535.7 ± 206.4 µU min/ml, P=0.312), 2ndIR increased significantly ( 64.7 ± 18.8 µU/ml *vs.* 70.9 ± 22.7 µU/ml, P < 0.01) by treatment. Pioglitazone therapy also demonstrated marked improvements of K and ISI values [1.75 ± 0.58 *vs.* 2.08 ± 0.62 (P < 0.01) and 0.120 ± 0.035 mg Kg$^{-1}$ min$^{-1}$/µU ml$^{-1}$ *vs.* 0.142 ± 0.047 mg Kg$^{-1}$ min$^{-1}$/µU ml$^{-1}$ ( P < 0.05), respectively].
**Conclusion:** In MS patients with IGT, pioglitazone therapy was associated with improvement of insulin secretion, suggesting ameliorated beta-cell dysfunction. Additionally, as the changes of K and ISI values may have shown, insulin sensitivity also improved compared with baseline.

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**Metabolic benefits of adding pioglitazone to patients with Type 2 diabetes (T2D) already optimized on insulin therapy**

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**Background and aims:** As T2D progresses, the underlying insulin resistance persists and insulin deficiency becomes more severe, often requiring exogenous insulin (INS). Some patients do not attain metabolic targets despite optimization of INS therapy. Adding pioglitazone (PIO), a PPAR-γ agonist that reduces insulin resistance, to the INS regimen of these patients may further improve their metabolic control. We compared the effect on glycemic and lipid control of adding PIO or placebo (PLB) to the INS regimen of patients with T2D already optimized on insulin therapy.
**Materials and methods:** In this multicenter, randomized, double-blind clinical trial, INS-requiring patients with T2D and hemoglobin A1C (A1C) ≥7.5% had INS regimens optimized over 3 months (the dose, type, and regimen of INS could be adjusted) to achieve fasting and preprandial blood glucose <5.5 mM and 2 hour postprandial blood glucose <7.5 mM. After optimization, patients with A1C ≥7.0% were randomized to PIO (30 mg/day) + INS (n = 142) or PLB + INS (n = 147) and treated for 6 months. Initially the INS dose was reduced 10% to prevent hypoglycemia. Throughout the 6-month treatment period, the INS dose could be adjusted to attain and maintain glycemic targets and prevent hypoglycemia. Fasting plasma glucose (FPG), A1C, fasting serum C-peptide, cholesterol (total, LDL and HDL) and triglycerides (TG) were measured at baseline and 6 months. Statistical analysis of covariance using least squared means model was performed.
**Results:** Baseline characteristics and demographics of the 2 groups were similar. Whereas at 6 months PIO + INS reduced mean A1c (-0.69%, p<0.001) and FPG (-1.45 mM, p<0.001) from baseline, PLB + INS did not. The between-treatment differences ([PIO + INS] - [PLB + INS]) for A1C (-0.55%, p<0.001) and FPG (-1.80 mM, p<0.0001) occurred despite a reduction of INS dose used in the PIO + INS group from baseline (-0.16 U/day*kg, p<0.001) and a between-group difference (-0.18 U/day*kg, p<0.001). The PIO + INS group also had a greater reduction in the C peptide (-79.7 pmol/L, p<0.05) vs the PLB + INS group. PIO + INS increased HDL-C (0.10 mM, p<0.001), reduced the ratio of geometric mean (PIO/PLB) of log transformed TG (p<0.05) and lowered TC/HDL-C (p<0.05) from baseline. There was no change in LDL-C or TC values. The between-treatment differences were: HDL (0.13 mmol/L, p<0.001) and TC/HDL-C (-0.38, p<0.005) and TG (0.87 p<0.05). Weight increase with PIO + INS was higher than for PLB + INS group. The hypoglycemia rate did not change from baseline for both groups and between groups at 6 months, but the subjective reporting of hypoglycemic episodes was greater for PIO + INS (n=40) than for PLB + INS (n=22) groups. Edema was reported more often with PIO + INS (n=20) than with PLB + INS (n=5), but no increase in cardiac events was noted.
**Conclusions:** Adding PIO to patients already optimized on insulin further improved glycemic control as evidenced by a significant lowering of A1C and FPG. PIO + INS also raised HDL-C, reduced TG and lowered TC/HDL-C ratio. In addition the daily insulin dose was reduced in the PIO group.

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**The effects of thiazolidinediones on left ventricular structure and function in Type 2 diabetic patients**

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**Background and aims:** Thiazolidinediones are widely used oral antihyperglycemic drugs that decrease insulin resistance but have also been associated with weight gain and edema. Pioglitazone (Pio) has been approved for use in Japan. The aim of this study is to determine whether and how the use of Pio affects left ventricular (LV) structure and function in type 2 diabetic patients.
**Materials and methods:** Twenty type 2 diabetic patients with no history of cardiac failure or heart disease were enrolled and received Pio (15~30 mg/day) for 6 months. Two patients dropped out because of skin eruptions and dizziness. An experienced blinded examiner performed M-mode and Doppler echocardiography on 18 type 2 diabetic patients (12 men, age (mean(SD)) 57(11) years, body mass index (BMI) 24 (3) kg/m$^2$) at baseline, 3 months, 6 months, and finally 12 months after the cessation of Pio treatment, which is 18 months from the baseline. M-mode parameters including LV end-diastolic diameter (LVDd), end-systolic diameter (LVDs), and ventricular septum thickness (SVTd) and posterior wall thickness in diastole (PWTd) were measured and LV mass index (LVMI) was calculated according to Penn's formula. LV diastolic function was assessed by the ratio between the peak diastolic velocity and the peak atrial systolic velocity (E/A).
**Results:** Body weight (BW) significantly increased in all type 2 diabetic patients at both 3-months and 6 months after treatment began (baseline 66.3(9.6), 3M 68.6(10.1), and 6M 69.5(11)kg, respectively) ($p<0.001$). LVDd increased (48.1(3.4), 50.4(3.4), 50.9(4.3)mm, respectively) ($p<0.01$), while LVDs, STd, PWTd, FS, and E/A did not change. HbA1c significantly decreased (7.4(0.9) vs 7.1(1.0) %, $p<0.01$) and serum BNP significantly increased (16.8(19.2) vs 21.4(18.9) pg/ml, $p<0.05$) between the baseline and 6 month stage of the trial. Blood pressure and hematocrit were about the same during the 6 month treatment period. Twelve months after the cessation of Pio treatment, BW and LVDd (67.4(10.5) kg, 49.0(3.0)mm) declined but did not return completely to baseline levels. A small statistically significant difference between baseline and 12-month post-treatment levels remained.
**Conclusion:** Body weight increases occurring during treatment with Pio may be caused by an increased circulation volume and is largely reversible with the cessation of Pio treatment in type 2 diabetic patients.

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**Rosiglitazone preserves islet beta cell function in non-insulin-dependent LADA patients**

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**Background and aims:** Latent autoimmune diabetes in adults (LADA), caused by the immune mediated destruction of islet insulin-secreting β cells, can serve as a human model for autoimmune type 1 diabetes. Except two trials with insulin, intervention studies aiming at preserving islet β cell function in LADA are limited. But most patients do not like shots when they still can be treated with oral agents. Rosiglitazone has the ability to down-regulate autoimmune reactions in vitro and to maintain beta cell mass in animal models. Thus, we hypothesized that rosiglitazone instead of a sulfonylurea agent would aid in the preservation of beta-cell function in LADA.
**Materials and methods:** LADA patients, defined as glutamic acid decarboxylase (GAD) antibody positive phenotypic type 2 diabetes, with a fasting C peptide of 0.3nmol/L or more and with duration less than 5 years, were enrolled and randomly assigned to receive sulfonylurea ( SUR group, n=18) or rosiglitazone (RSG group, n=19). Blood was drawn every 6 months to determine plasma glucose, HbA1c and C peptide at fasting (FCP) and 2 hours after taking 75 g glucose (PCP) without medication for comparison. Islet beta cell function and insulin resistance were calculated with HOMA formula, and labeled as HOMA-IS and HOMA-IR respectively. GAD antibody and C peptide were measured with radioimmune assay.
**Results:** All of the 37 patients have been followed up for 6 months, 32 cases for 12 months and 19 for 18 months. (1) After six months' follow-up, HOMA-IR and FCP levels in patients treated with rosiglitazone decreased significantly, while HOMA-IS decreased significantly in patients treated with sulfonylurea, from 135.9 ± 97.5 to 100.5 ± 39.2 mU/mmol. (2)During the 12 months' observation, HOMA-IR index decreased significantly (4.0 vs 7.0, $P<0.05$) in RSG group, without changes for HOAM-IS, while the value of HOMA-IS decreased in SUR group (97.2 vs 107.3, $P<0.05$). (3) As for patients treated for 18 months, PCP (4.32 vs 1.99 nmol/L, $P<0.05$) and C peptide response (ΔCP) levels (3.47 vs 1.34 nmol/L, $P<0.05$) in RSG group were higher than those in SUR group. (4)With HOMA-IS level at 6th month as dependent variable and different treatment, age at onset, disease duration, BMI, GAD-Ab index, HbA1c, FCP, PCP, HOMA-IR and HOMA-IS levels at entry as independent variables, the multiple stepwise regression analysis showed that only GAD antibody titer and different treatment regime entered the regressive equations, with standard regression coefficients 9.24 ($P=0.000$) and 2.51 ($P=0.019$) respectively.
**Conclusion:** Rosiglitazone, instead of sulfonylurea, could preserve islet β cell function in non-insulin-dependent LADA patients.
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**Thiazolidinediones, like exercise, acutely stimulate uncoupling protein-3 mRNA expression in rat skeletal muscle**

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**Background and aims:** Thiazolidinediones (TZDs) are believed to induce insulin sensitization via the activation of peroxisome proliferator-activated receptor-gamma (PPARgamma). *In vitro*, however, they have immediate effects on skeletal muscle that are not mediated by PPARgamma and resemble the rapid effects of contractions and hypoxia (e. g., a reduction in cellular ATP and an increase in glycolysis). To study whether TZDs have rapid exercise-like and hypoxia-like actions also *in vivo*, we examined the acute effects of a single TZD injection on UCP-3 mRNA expression in skeletal muscle and on circulating plasma free fatty acids (FFA), both of which are known to increase immediately in response to exercise and hypoxia.
**Materials and methods:** Healthy male rats received a single intraperitoneal injection of the TZDs pioglitazone, rosiglitazone, or RWJ-241947 (also known as MCC-555). Control rats were injected with the vehicle only. After 2 h or 6 h, rats were killed and samples of muscle tissue were collected for the quantification of UCP-3 mRNA. Plasma was sampled to measure FFA concentrations. In an additional experiment, isolated specimens of rat muscle were exposed to pioglitazone *in vitro* in order to examine the direct effects of the TZD on skeletal muscle in the absence of increased ambient FFA.
**Results:** A single intraperitoneal TZD injection stimulated UCP-3 expression in gastrocnemius muscle within 2 h (225 µmol/kg pioglitazone: +125 ± 40%, $p<0.05$; data given as % increase *vs* vehicle-treated controls). After 6 h, the increase in UCP-3 mRNA was even more distinct and independent of the individual TZD injected (50 µmol/kg TZD: pioglitazone, +935 ± 319%, $p<0.03$; rosiglitazone, +769 ± 119%, $p<0.001$; RWJ-241947, +848 ± 271%, $p<0.03$). The increases in UCP-3 mRNA were accompanied by elevated plasma FFA (control, 158 ± 13 µmol/l; *vs* pioglitazone, 281 ± 40 µmol/l, $p<0.03$; *vs* rosiglitazone, 276 ± 27 µmol/l, $p<0.005$; *vs* RWJ-241947, 398 ± 51 µmol/l, $p<0.004$). Furthermore, TZD-induced stimulation of UCP-3 expression was found independently of the muscle fibre composition, although it was less pronounced in red than in white muscle (75 µmol/kg pioglitazone: +364 ± 53% in white tibialis anterior muscle; but only +61 ± 21% in red soleus muscle; $p<0.05$ each). The observed rapid responses to TZD treatment thus strongly resembled those reported in response to a bout of exercise or hypoxia. The elevation in plasma FFA could in part have contributed to TZD-induced UCP-3 expression, but an increase in UCP-3 mRNA was likewise observed in isolated specimens of tibialis anterior muscle after 2 h of pioglitazone exposure *in vitro*, *i.e.* without any change in ambient FFA (25 µmol/l pioglitazone: +73 ± 34%, $p<0.05$).
**Conclusion:** TZDs immediately stimulate the expression of UCP-3 mRNA in skeletal muscle of conscious rats, which at least in part seems to be due to a direct and FFA-independent mechanism of action. Our findings show that TZDs have rapid exercise-like and hypoxia-like effects not only *in vitro* but also *in vivo*, which corroborates the hypothesis that such actions could contribute to their delayed insulin-sensitizing effects.

# OP 27

# Coronary heart disease: risk factors and epidemiology

## 157

**Could non-HDL cholesterol replace total/HDL cholesterol ratio to estimate coronary heart disease risk in the UKPDS risk engine?**

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**Background and aims:** The UKPDS Risk Engine is a diabetes-specific coronary heart disease (CHD) risk calculator for use in patients without known CHD. It estimates CHD risk (fatal and non-fatal myocardial infarction including sudden cardiac death) using conventional CHD risk factors, $HbA_{1c}$, duration of diabetes and total/HDL cholesterol ratio. It has been suggested that non-HDL cholesterol (total cholesterol - HDL cholesterol) might be a simpler measure than the total/HDL cholesterol ratio and might better reflect CHD risk. We have constructed a UKPDS Risk Engine model that uses non-HDL cholesterol to compare with that using the total/HDL cholesterol ratio.
**Materials and methods:** 4,540 of 5,102 UKPDS patients with type 2 diabetes had sufficient data for this analysis. Survival models for CHD risk, adjusted for age, sex, race, smoking status, $HbA_{1c}$, and systolic blood pressure, were developed that adjusted also for total/HDL cholesterol or non-HDL cholesterol. Model fit was measured by Akaike's Information Criterion, with higher values indicating better fit. To determine whether differences in Akaike's Information Criterion correspond to statistically significant differences between models, we also conducted likelihood ratio tests of the two models against a reference model that contained both total cholesterol and HDL cholesterol.
**Results:** 517 CHD events occurred during 29,878 person-years of follow-up. Akaike's Information Criterion was 9.20 and 3.76 for total/HDL cholesterol and non-HDL cholesterol respectively, indicating that total/HDL cholesterol is a better predictor of CHD risk than non-HDL cholesterol. The total/HDL cholesterol model was equivalent to the reference model ($p=0.38$) but the non-HDL cholesterol model was significantly worse ($p<0.0001$). The risk ratio for total/HDL cholesterol was 1.23 (95% confidence interval 1.17-1.29) permmol/L.
**Conclusions:** Total/HDL cholesterol is a superior measure of CHD risk than non-HDL cholesterol. It captures the protective effect of HDL cholesterol as well as the harmful effects of non-HDL cholesterol in a single parameter and remains the dyslipidaemic measure of choice in the UKPDS Risk Engine (www.dtu.ox.ac.uk/riskengine).
*Supported by: the Healthcare Foundation. Funding for the UK Prospective Diabetes Study has been listed previously (Lancet 352 page 852).*

## 158

**Prevalence and predictors of silent myocardial ischaemia in Type 2 diabetes detected by electron beam CT and myocardial perfusion imaging**

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**Background and aims:** Myocardial infarction remains a major cause of death in type 2 diabetes and often occurs in patients with no previous cardiac symptoms. Early detection of coronary artery disease could therefore have considerable impact on diabetes management, enabling early intervention and possible prevention of events. We therefore studied the efficacy of coronary artery calcium (CAC) imaging by electron beam tomography (EBT) combined with myocardial perfusion imaging in detecting asymptomatic coronary artery disease and silent myocardial ischaemia (SMI) in patients with type 2 diabetes.
**Materials and methods:** 400 consecutive asymptomatic type 2 patients aged 30–65 with no cardiovascular history and normal ECG were recruited from a hospital diabetic clinic. All underwent EBT (using C150 GE IMatron scanner) and the extent of coronary calcification was quantified by calculation of Agatston scores. Those with significant coronary atherosclerosis (CAC score >100 Agatston units) were further evaluated by stress/rest ECG gated Tc99 m-sestamibi perfusion imaging to determine the extent of SMI. Summed Stress scores (SSS) were calculated to quantify the extent of ischaemia
**Results:** Subjects had a mean age of 56 ± 7 years, mean diabetes duration of 10 ± 7 years and mean HbA1c of 8 ± 1.7%. Significant atherosclerosis (CAC>100) was demonstrated in 100 subjects (25%). Of these 100 subjects, 48 had abnormal MPI (SSS>4) and 20 had markedly abnormal MPI (SSS>7). Perfusion abnormalities increased in frequency with increasing CAC scores and were present in 67% of those with CAC>400. Age, male sex, smoking, history of hypertension and duration of diabetes were independent predictors of subclinical atherosclerosis in a multivariate stepwise linear regression model. The extent of coronary artery calcification and the duration of diabetes were the strongest determinants of the presence of silent ischaemia.
**Conclusion:** These data demonstrate the presence of significant subclinical atherosclerosis and SMI in an alarmingly high proportion of asymptomatic type 2 diabetic subjects. The extent of CAC detected by EBT predicts SMI and EBT may therefore prove useful as a screening tool for non-invasive assessment of coronary risk in subjects with type 2 diabetes.

## 159

**Abnormal glucose regulation in patients with coronary artery disease across Europe**

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**Background and aims:** The presence of diabetes mellitus (DM) and impaired glucose tolerance (IGT) is of substantial prognostic importance for patients (**P**) with coronary artery disease (CAD). The aim was to assess the prevalence of DM and IGT in **P** with CAD.
**Materials and methods:** A survey was conducted between Feb 2003–Jan 2004 as a multicenter prospective observational study involving 110 centers in 25 countries. Consecutive **P** aged >18 years were screened for a diagnosis of CAD when admitted to the participating hospital wards or outpatient clinics. Data on demography, medical history, treatment, reason for admission, clinical status at enrolment and final diagnosis were collected by means of a web based electronic case record form. Fasting plasma glucose (FPG) and 2 hours post load glycemia were requested in all **P** without known DM. OGTT was performed when **P** was in a stable clinical condition. Glucometabolic status was classified as normal (N), impaired fasting glucose (IFG), IGT or DM.
**Results:** 4 961 **P**, with previously (72%) or newly (28%) diagnosed CAD were enrolled while admitted to the hospital on acute (2107) or scheduled (1137) basis or enrolled at an outpatient clinic (1717). **P** 71% men, aged 65 ± 11 years, were split according to the clinical condition into **Acute** (**A**: 2107) and **Chronic** (**Ch**: 2 854) cohort. The CAD was diagnosed previously in 50% **A** and 89% **Ch**. DM was already known in 1524 (31%) **P**. A history of myocardial infarction and heart failure were reported in **A** (33%, 19%) and **Ch** (53%, 25%) **P** respectively. **A** were admitted due to: acute myocardial infarction (56%) and/or heart failure (14%). **Ch** were included during elective hospitalisation (29%) or visit at the outpatient clinic (71%). OGTT results are shown in a table. When glucometabolic state was assessed by FPG only, two thirds of **P** with abnormal glucose regulation remained undiagnosed (**A**: 338 out of 534; **Ch**: 327 out of 511). Assuming that OGTT outcome would have been similar among all **P** and accounting for those with known DM, a complete pattern of glucose tolerance was estimated (N / IGT / DM) respectively: **A** - 29%/ 32%/ 46% and **Ch** - 34%/ 32%/ 40%.
**Conclusion:** This Survey demonstrates that normoglycemia in fact is less common than abnormal glucose regulation among patients with CAD. FPG has a limited capacity to identify subjects with truly abnormal glucose metabolism. An OGTT should be included in the diagnostic routines for accurate evaluation of total cardiovascular risk in high risk individuals and especially in patients with CAD.

Glucose regulation assessed by an OGTT

| Clinical condition | Normal | IFG | IGT | Diabetes |
|---|---|---|---|---|
| **Acute** *(n=923)* | 389 (42%) | 39 (4%) | 294 (32%) | 201 (22%) |
| **Chronic** *(n=997)* | 486 (49%) | 50 (5%) | 320 (32%) | 141 (14%) |

## 160

**Depressive symptoms predict all-cause and cardiac mortality in Type 2 diabetic subjects: The Fremantle Diabetes Study**

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**Background and aims:** Clinical depression may be an independent risk-factor for poor outcomes in patients with coronary heart disease, and the prevalence of depression and depressive symptomatology in diabetic populations is roughly double that seen in the community. We wished to determine whether patients with type 2 diabetes with co-existent depressive symptoms had increased cardiac mortality.
**Materials and methods:** 1273 patients with type 2 diabetes from a community-based longitudinal observational study, who had completed the self-administered General Health Questionnaire (GHQ), were followed for 7.8 ± 2.4 years from study entry. Using multiple logistic regression analysis and Cox proportional hazards models, we investigated the potential impact of severe-degree (based on visual-analog scales) self-rated depression symptoms from the GHQ at baseline on all-cause and cardiac mortality obtained from the Western Australian Death Register.
**Results:** Depressive symptoms were common at baseline (53.7% reported none, 14.8% reported one, 31.5% reported 2 or more symptoms). By June 2003, 361/1273 subjects (28.4%) had died and 152 (42.1%) deaths were due to cardiac causes. After adjustment for univariate factors with $P<0.05$ (age, gender, diabetes duration, BMI, systolic BP, $HbA_{1c}$, diabetes treatment, diabetic complications, smoking, marital status, education), the presence of 2 or more depressive symptoms was independently associated with all-cause mortality (odds ratio (95% confidence limits): 1.49 (1.06, 2.09); $P=0.022$) and with cardiac death (1.52 (1.00, 2.31); $P=0.048$). Cox modelling revealed time-dependency in the effect of depression symptoms on risk of cardiac death, with a greater risk of cardiac death in patients with depressive symptoms only apparent 2 years post-baseline.
**Conclusions:** The presence of depressive symptoms in patients with type 2 diabetes is an important independent predictor of all-cause and cardiac-related death. Therapy for depression may have a beneficial impact on prognosis in diabetes and should be further investigated.
*Supported by: Raine Foundation, Western Australia*

## 161

**Myocardial infarction in Type 1 diabetes – a population-based follow-up study**

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**Background and aims:** To determine the incidence of myocardial infarction and its associated factors in type 1 diabetes within a geographically defined population.
**Materials and methods:** The original records of all the 1132 patients with onset of type 1 diabetes below age 40 diagnosed in the Erfurt district from 1966 to 1988 were analysed up to the end of 1990. This represents more than 12600 diabetes-years.
**Results:** Myocardial infarction occured after 11 ± 6 years of diabetes at 40 ± 11 years of age. The cumulative incidence (life table analysis) amounted to 5.0% after 25 years of diabetes and 12.1% by age 55 without significant differences between men and women. The earliest case precipitated at age 20. The patients with myocardial infarction (n = 15) had a higher BMI (25.4 ± 5.0 vs 24.2 ± 3.5 kg/m$^2$) and a higher systolic blood pressure (147 ± 21 vs 136 ± 16 mmHg) during the first 15 years of diabetes in comparison ($p < 0.05$) to the remaining patients of this cohort matched for sex (27% women), age (42 ± 11 vs 42 ± 7 years) and duration of diabetes (16 ± 5 vs 16 ± 6 years) at the end of follow-up (n = 393). A higher diastolic blood pressure was statistically significant during the first 5 years of diabetes only (90 ± 7 vs 83 ± 8 mmHg). Patients with later myocardial infarction needed more insulin during the first 10 years of diabetes (significantly different at diabetes onset only) despite similar blood glucose levels during the same period of time. More of them developed proteinuria (53 vs 21%), chronic renal failure (20 vs 5%), hypertension (80 vs 33%), and claudication (27 vs 9%), underwent a major leg amputation (13 vs 2%), and died (40 vs 8%) during the follow-up period. A threefold rate of stroke (7 vs 2%) did not reach statistical significance. There was no substantial difference with respect to background (73 vs 58%) and proliferative retinopathy (7 vs 5%).
**Conclusion:** Myocardial infarction in type 1 diabetes followed a higher body mass index, increased blood pressure, as well as a higher need of insulin and was associated with a higher risk of hypertension, peripheral vascular disease, nephropathy, and early death. This cluster is similar to the metabolic syndrome known from type 2 diabetes. Hence the especially poor prognosis of some of the patients with type 1 diabetes may be due to the concomitant presence of the insulin resistance syndrome.

## 162

**Mortality and cardiovascular morbidity in Type 1 diabetic patients with nephropathy - 10 years prospective follow-up study**

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**Background and aims:** In early studies of the natural course of diabetic nephropathy a median survival time of 5-7 years from onset of diabetes was observed. Furthermore end stage renal disease (ESRD; serum creatinine ≥ 500 µmol/L) was the main cause of death (approximately 65% of patients). We therefore assessed the impact of long term renoprotective treatment on prognosis and cardiovascular morbidity and mortality in diabetic nephropathy.
**Materials and methods:** In a prospective observational follow-up study 199 type 1 diabetic patients with overt nephropathy (122 men, age (mean(SD)) 41 ± 10 years, duration of diabetes 28 ± 8 years, GFR 74 ± 34 mL/min/1.73 m$^2$ ) and a matched control group of 192 patients with normoalbuminuria (118 men, age 43 ± 10 years, duration of diabetes 27 ± 8) were followed for 10 years.
All proteinuric patients received antihypertensive treatment with predominantly ACE-inhibitors or angiotensin II receptor blockers combined with diuretics aiming at a blood pressure below 140 mmHg systolic and 90 mmHg diastolic.
The primary endpoint was a composite endpoint of cardiovascular death, hospitalization for myocardial infarction or stroke, coronary artery bypass grafting or percutanous transluminal coronary angioplastic, ischaemic amputation or peripheral bypass-surgery. Secondary endpoints were all cause mortality, cardiovascular mortality and death due to ESRD defined as death in a patient with serum creatinine ≥500 µmol/L within the last year prior to death independently of the cause of death.
**Results:** During 10 years of follow-up 79 (40%) patients with diabetic nephropathy reached the primary endpoint versus only 19 (10%) of normoalbuminuric patients (log rank test; $p<0.001$). 60 (30%) patients with nephropathy died versus 16 (8%) with normoalbuminuria. In patients with nephropathy 25 (42%) deaths were ascribed to cardiovascular causes versus 7 (38%) deaths in normoalbuminuric patients. ESRD was the cause of death in 30 patients (50%) with overt nephropathy.
Cox multiple regression analysis identified the following significant predictors of the primary endpoint: nephropathy (relative risk 3.03; 95% confidence interval 1.70 to 5.39), earlier cardiovascular event (3.05; 1.93 to 4.84), 10 year increase of age (1.28; 1.05 to 1.56), and 10 mmHg increase in systolic blood pressure (1.13; 1.03 to 1.25). Sex, smoking, HbA1 c, and total cholesterol were not significant predictors in the final model of the primary endpoint.
**Conclusion:** The survival of patients with diabetic nephropathy has improved considerably. However diabetic nephropathy is still a major risk factor for cardiovascular morbidity and mortality in type 1 diabetic patients. Consequently our study shows that the future focus must be on multifactorial treatment aiming at preventing cardiovascular disease.

# OP 28

# Clinical nephropathy and epidemiology

## 163

**Blunted nocturnal blood pressure reduction predicts mortality in Type 2 diabetic patients: 14 years follow up study**

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**Background and aims:** To evaluate the prognostic significance of ambulatory blood pressure level and nocturnal blood pressure reduction, left ventricular hypertrophy, presence of diabetic nephropathy and conventional risk factors for all cause mortality in Type 2 diabetic patients.
**Materials and methods:** Fourteen year observational follow up study of Type 2 diabetic patients: 51 subjects with overt diabetic nephropathy (albuminuria >=300 mg/24 h) and 53 normoalbuminuric diabetic controls (<30 mg/24 h) mean age (SD) 60 (8)years, 24/80 female/males. At baseline we measured left ventricular hypertrophy LVH (echocardiography) ambulatory blood pressure (Takeda TM2420), glomerular filtration rate GFR (51Cr-EDTA) autonomic neuropathy (heart rate variability upon deep breathing BTB) in addition to conventional risk markers for mortality. Nocturnal blood pressure reduction (dip) was defined as the relative change in daytime to nighttime ambulatory blood pressure.
**Results:** During follow-up 54 of 104 patients died. Cox multiple regression analysis identified the following significant predictors of all cause mortality: male sex (relative risk 2.59; 95% confidence interval 1.31 to 5.12) age (year) (1.06; 1.02 to 1.11) GFR (ml/min/1.72 m2)(0.985; 0.97 to 0.997), BTB (0.92; 0.87 to 0.98), daytime systolic blood pressure (mmHg)(1.02; 1.01 to 1.03), dip(%)(0.97; 0.94 to 0.998) presence of LVH (2.54; 1.30 to 4.94) and haemoglobin A1 c (1.15; 0.99 to 1.34). Albuminuria could replace GFR in the model. Smoking, lipids, known diabetes duration, BMI, daytime diastolic pressure, and office blood pressure were excluded from the model.
**Conclusion:** In addition to established risk factors for mortality (LVH, impaired renal function, hypertension, poor glycaemic control and autonomic neuropathy) blunted nocturnal blood pressure reduction predicts increased mortality in Type 2 diabetic patients with and without diabetic nephropathy.

## 164

**Increased subclinical atherosclerosis in Type 2 diabetic patients with microalbuminuria**

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**Background and aims:** Microalbuminuria appears to be a risk marker for atherosclerosis. However, little is known about the direct association between microalbuminuria and vascular wall properties. The aim of the study was to investigate the relationship between microalbuminuria and markers of vascular wall properties including carotid intima-media thickness (IMT) and pulse wave velocity (PWV) in type 2 diabetic patients.
**Material and methods:** Subjects were 306 type 2 diabetic patients with normoalbuminuria (n=200) and microalbuminuria (n=106). Those who had macroalbuminuria, atherosclerotic vascular disease, and/or ankle brachial index being less than 0.9 were not included. Brachial-ankle PWV was measured by automatic oscillometric method. IMT of the common carotid artery was measured using high-resolution B-mode ultrasonography and a novel computerized image-analyzing system.
**Results:** Average IMT, maximum IMT, and PWV were significantly higher in patients with microalbuminuria than in patients with normoalbuminuria. Both average and maximum IMT increased significantly as albuminuria increased in the microalbuminuric range. Average IMT and maximum IMT correlated significantly with PWV ($p<0.0001$), although some patients exhibited increased levels of only PWV or IMT. By a multiple linear regression, age and albuminuria were independent predictors of IMT and PWV. Waist circumference was an independent predictor of IMT. Hypertension and $HbA_{1C}$ were independent predictors of PWV. After adjustment for conventional cardiovascular risk factors including age, sex, waist circumference, $HbA_{1C}$, hypertension, hyperlipidemia, and smoking, albuminuria revealed a significant association with average IMT, maximum IMT, and PWV ($p<0.05$, $p<0.0001$, and $p<0.05$, respectively).
**Conclusion:** A slight elevation of albuminuria is a significant determinant of IMT and PWV independent of conventional cardiovascular risk factors in type 2 diabetic patients with no clinical nephropathy nor any vascular diseases. This significant association might point to a link in the pathogenesis of atherosclerosis and diabetic nephropathy.

## 165

**Increasing incidence of proteinuria in Pima Indians with Type 2 diabetes**

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**Background and aims:** To determine if the incidence of clinical proteinuria has declined in response to the widespread use of medicines to treat diabetic kidney disease, or if changes in incidence are attributable to changing demographic characteristics of the diabetic patients, trends in the incidence rate of proteinuria in three 12 3/4-year time intervals between 1965 and 2003 were examined in diabetic Pima Indians.
**Materials and methods:** The study included 1,442 type 2 diabetic subjects (510 men and 932 women) aged ≥25 years from the Gila River Indian Community. Incidence rates of proteinuria (in cases/1000 person-years) were computed independently in the three time periods. Proteinuria was defined as a urinary protein-to-creatinine ratio ≥0.5 g/g.
**Results:** 411 subjects developed proteinuria during a median follow-up of 5.4 years (range = 0.3-12.4 years). The overall age-sex-adjusted incidence of proteinuria increased from 22 cases/1000 person-years (95% CI, 16–27) in 1965–77 to 36 cases/1000 person-years (95% CI, 28-44) in 1990–03 ($p_{trend}=0.0004$). The age-sex-adjusted duration-specific incidence rates of proteinuria, however, were similar in each duration group during the study period ($p_{trend}=0.31$).

| Study Periods | Duration of Diabetes (years) 0–4 | 5–9 | 10–14 | 15–19 | Age-sex-duration adjusted IRR* |
|---|---|---|---|---|---|
| 2/1965-10/1977 | 7.0 (2.5–11) | 19 (9.9–29) | 63 (28–99) | 87 (33–142) | 1.0 |
| 11/1977-6/1990 | 7.8 (2.7–13) | 18 (7.4–29) | 46 (29–62) | 120 (63–178) | 0.97 (0.72–1.3) |
| 7/1990-2/2003 | 7.9 (2.9–13) | 33 (14–52) | 51 (30–72) | 107 (69–145) | 1.1 (0.83–1.5) |

Age-sex-adjusted incidence rates per 1000 person-years (95% CI).
* IRR = incidence rate ratio relative to the first time period.

**Conclusion:**The incidence of proteinuria among Pima Indians of similar age, sex, and duration of diabetes has remained largely unchanged during the course of this study despite the widespread use, especially in the 1990's, of medicines that block the renin-angiotensin system, lower blood pressure, and improve glycemic control. This finding suggests that other, more effective treatments are required if we are to control the worldwide epidemic of diabetic kidney disease.

## 166

**The worrying issue of diabetic nephropathy in Canarian Islands: epidemiological study and possible causes**

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**Background and aims:** Diabetes Mellitus (DM) has become the most common single cause of end-stage renal disease (ESRD) in the U.S. and Europe. In the Canarian Islands diabetic nephropathy (DN) accounts for about 54% of new cases of ESRD with 78.2 new cases per million of persons and year (y) it is probably one of worrying issue that remain unresolved in Canarian healthsytem. This data are three times greater than the mean of Spain.
**Materials and methods:** A prospective, registry based, observational study of all diabetic patients in ESRD in Tenerife (Canary Islands) over a 2-year period. A validated survey of 115 questions was applied to 253 patients in ESRD by DN in our studied area (900.000 inhabitants) and a control group of 65 patients in ESRD without diabetes. The items included familiar records, socioeconomic status, rural/ urban area residence and issues of educational and clinical interventions.
**Results:** Of 318 patients who were studied, the DM group -169 t2DM, 85 t1DM- have a mean age of 60.6 ± 12 y; Males: 60,5%; t2DM: 72,6%; time of evolution of diabetes: 20.9 ± 10 y. Comparisons included 65 sex and age-matched ESRD controls.

We hypotesized the possible factors involved in the wide differences differences in health status of diabetics between spanish regions:
Prevalence: we cannot explain the differences by the slight increase of the prevalence of Diabetes in Canarian people (6–10% in Spain vs. 8–12% in Canary Islands).
Genetic origin: 87% of the DM they were native Canarian (both parents, 4 grandparents Canarian and without well-known ancestors outside of the islands). Only 79% of the t1DM were native Canarian. It did not exist difference between the t2DM (90%) and the control group .
Rural/ urban ratio: 62% of the sample lives in rural area vs. to 33% of the general population. No difference with the controls existed. High pecentge (39%) of the rural ones lived in small area below 1000 inhabitants.
Territorial aggregation: In 51% of DM group, the parents and the 4 grandparents proceeded of the same area. 21% additional proceeded of a territory of less than 20 km around.
Familiar aggregation: 40,6% t2DM had 2 or more diabetic siblings. 33% of siblings were diabetics.
Sociocultural level: the incomes for family unit in the diabetics were inferior significantly (40% below poverty level) than the general population and the controls. The same thing happened with the level of studies. 47% of the DM group were illiterate or they did not finish the primary studies.
Previous medical control: the multiple obtained data allow to assure that the system effectiveness for the detection precocious of DM and their complications, later control and educational information to the persons with diabetes was very inappropriate.
Diet: The ENCA (Wide and complete nutritional study of canarian population made in 1999) showed very inadequate nutritional habits with high use of sugar and derivatives (122% above) and poor fruits and vegetables consume (39% minor) in relation to mean spanish pattern.
**Conclusion:** Our group in ESRD for DN are fundamentally Canarian native, of rural area, socially disadvantaged, with intense territorial and familiar aggregation, with harmful diet and inadequate previous metabolic control. It becomes possible that the combination of genetic factors, socioeconomical conditions and a bad metabolic control can explain the high prevalence of ESRD due diabetes in the Canarian Islands.
*Our acknowledgement regarding support we have received for the study from MSD*

## 167

**Prevalence and risk factors for microalbuminuria in Type 2 diabetic patients: A global perspective**
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**Background and Aims:** To describe the characteristics of type 2 diabetic patients in the Developing Education on Microalbuminuria for Awareness of Renal and Cardiovascular risk in Diabetes (DEMAND) study evaluating the global prevalence and determinants of microalbuminuria (MA). Furthermore, cardiovascular protective treatments were audited.
**Materials and Methods:** Cross-sectional study evaluating 31470 type 2 diabetic patients without known albuminuria from 34 different countries. Albumin/creatinine ratio in a random urine specimen was successfully measured in 86.5% (N=27470) of the DEMAND patients. At the screening carried out between June and September 2003 we recorded demographic (white 47%, Asian 42%, Hispanic 6%, black 2%, other 3%), clinical (mean age 61 years, known diabetes duration 8 years, history of cardiovascular diseases 30%, smoking 26% and hyperlipidemia 44%) and laboratory variables including drug treatment.
**Results:** The overall global prevalence of normo-, micro- and macroalbuminuric was 49%, 47.8% and 3.1%, respectively. The Asian and Hispanic patients had the highest prevalence of raised urinary albumin/creatinine ratio and the white the lowest. Age, diabetes duration, smoking, blood pressure elevation, HbA1 c, serum creatinine, vascular disease and left ventricular hypertrophy were all independent determinants of MA. Estimated GFR (Cockroft & Gault) was < 60 ml/min in 25% of the patients. Blood pressure below 130 mmHg was found in 33% and 44% had a HbA1 c below the recommended 7%. The frequency of patients ratio receiving baby aspirin was 40%, statins 31% and blood pressure lowering therapy 72%.
**Conclusion:** A high prevalence globally of microalbuminuria and reduced kidney function conditions associated with a enhanced renal and cardiovascular risk was detected in type 2 diabetic patients. Early detection and more aggressive multifactorial treatment aiming at renal and vascular protection are urgently needed.
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**Metabolic syndrome in type 1 diabetes: Association with diabetic nephropathy and glycaemic control (the FinnDiane Study)**
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**Background and Aims:** Insulin resistance and other components of the metabolic syndrome (MetS) have been implicated in the pathogenesis of both diabetic nephropathy and cardiovascular disease. Although, the MetS is a common feature, its presence in type 1 diabetes is largely unknown. The aim was therefore to estimate the prevalence of the MetS in Finnish type 1 diabetic patients and to assess whether it is associated with diabetic nephropathy and/or poor glycaemic control.
**Materials and Methods:** 2415 type 1 diabetic patients participating in the ongoing, nation-wide, multi-centre FinnDiane study (51% males, age 37 ± 1 yrs and duration of diabetes 22 ± 1 yrs). The MetS was defined according to NCEP ATPIII diagnostic criteria. By definition, all patients fulfilled the criteria for hyperglycaemia and three out of five criteria were required for diagnosis of the MetS. Based on their AER or medical files patients were classified as having normal AER (N=1261), microalbuminuria (N=326), macroalbuminuria (N=383) or ESRD (dialysis or transplantation, N=164). The glycaemic control was based on one measurement classified as good if $HbA_{1C}$ <7.5%, intermediate 7.5-9.0% or poor >9.0%.
**Results:** The overall prevalence of the MetS was 38% in males and 40% in females. More than four of the diagnostic criteria were observed in 14% of the patients. The prevalence of the MetS was 28% in those with normal AER, 44% in micro-, 62% in macroalbuminuric patients and 68% in patients with ESRD (p<0.001). The association between glycaemic control and presence of the MetS was:

| $HbA_{1C}$ (%) | <7.5 N=578 | 7.5–9.0 N=1087 | >9.0 N=695 | p-value |
|---|---|---|---|---|
| Metabolic syndrome | 31% | 36% | 51% | <0.001 |
| BP ≥130/85 mmHg or AHT | 60% | 71% | 76% | <0.001 |
| Waist >102/88 cm | 10% | 16% | 21% | <0.001 |
| Triglycerides ≥1.7 mM | 13% | 16% | 28% | <0.001 |
| HDL cholesterol <1.0/1.3 mM | 34% | 33% | 40% | 0.01 |

The frequency of MetS increased in parallell with hypertension when patients with good control were compared with those with intermediate glycaemic control. On the other hand, the higher frequency of MetS in patients with poor compared to intermediate glycaemic control was mostly due to an increase in dyslipidaemia. When patients with normal AER were analyzed separately regarding $HbA_{1C}$, a similar pattern could be observed.
**Conclusion:** Metabolic syndrome, as defined by NCEP criteria, is a frequent finding in type 1 diabetes and increases with more advanced diabetic nephropathy and worse glycaemic control. Whether this represents the metabolic syndrome as seen in type 2 diabetes is an open question.

# OP 29

# Microvascular complications in experimental models

## 169

**Ablation of the p66$^{Shc}$ longevity gene provides protection towards experimental diabetic glomerulopathy in mice**

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**Background and aims:** Ablation of p66$^{Shc}$ adaptor protein was shown to confer resistance to oxidative stress both *in vivo*, associated with a 30% increase in lifespan, and *in vitro*, together with protection from oxidative stress-induced p53-dependent apoptosis (Migliaccio E. et al., *Nature*, 402:309, 1999; and Trinei M. et al., *Oncogene*, 21:3872, 2002). Previous studies indicated that deletion of the p66$^{Shc}$ longevity gene protects from systemic and tissue oxidative stress, vascular cell apoptosis, and early atherogenesis in mice fed a high-fat diet (Napoli C. et al., *PNAS USA*, 100:2112, 2003), thus suggesting that this pathway may be implicated in vascular diseases associated with oxidant injury, including micro and macrovascular complications of diabetes. Aim of this study was to verify the hypothesis that deletion of the p66$^{Shc}$ longevity gene protects mice from the development of diabetic glomerular disease.
**Materials and methods:** p66$^{Shc}$ knockout (KO) mice and coeval wild type (WT) mice were rendered diabetic by i.p. injection of streptozotocin and killed 4 months later, together with their corresponding nondiabetic controls for the assessment of kidney function (serum creatinine - by the Jaffe method - and proteinuria - by the Bradford method) and structure (morphometric evaluation of glomerular and mesangial area), together with serum AGE levels (by ELISA) and renal content of the oxidation product 4-hydroxy-2-nonenal (HNE, by immunohistochemistry) and expression of p66$^{Shc}$ and fibronectin (by Western blot).
**Results:** Diabetic KO mice showed significantly less marked changes in renal function and structure vs. the diabetic WT animals, as indicated by the significantly lower levels ($p<0.001$) of proteinuria ($4.9 \pm 1.0$ vs. $13.9 \pm 2.1$ mg/mg creatinine), mean glomerular area (mGA, $3,228 \pm 183$ vs. $3,694 \pm 157$ mm$^2$) and mesangial fractional area (MFA, $24.5 \pm 2.7$ vs. $32.6 \pm 2.2\%$). Serum AGE levels were also less elevated in the diabetic KO vs. WT animals ($7.6 \pm 1.3$ vs. $12.6 \pm 1.4$ U/ml, $p<0.001$), despite similar increases in blood glucose ($20.4 \pm 1.9$ vs. $20.1 \pm 1.6$ mmol/l) and glycated hemoglobin ($13.4 \pm 0.8$ vs. $13.2 \pm 1.0\%$). Glomerular HNE immunoreactivity was detectable only in the diabetic WT mice, which showed also increased renal p66$^{Shc}$ expression vs. the nondiabetic WT mice. In addition, mGA ($2,515 \pm 176$ vs. $2,751 \pm 151$ mm$^2$, $p<0.05$), MFA ($22.0 \pm 1.6$ vs. $24.9 \pm 1.1\%$, $p<0.05$), proteinuria ($2.8 \pm 0.3$ vs. $3.3 \pm 0.5$ mg/mg creatinine, p=NS) and serum AGEs ($2.5 \pm 0.3$ vs. $3.9 \pm 0.7$ U/ml, p=NS) were slightly lower in nondiabetic KO than WT mice. Kidney cortex fibronectin expression showed the same trend, whereas serum creatinine did not differ among experimental groups.
**Conclusion:** These data indicated that resistance to oxidative stress is associated with at least partial protection from the development of diabetic glomerular disease and also with less marked renal injury induced by aging.

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**Imatinib attenuates diabetic nephropathy in apolipoprotein E-knock out mice**

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**Background and aims:** In the diabetic kidney, clinical as well as experimental observations have shown an up-regulation of growth factors such as platelet-derived growth factor (PDGF). These studies, however, were not designed to address whether up-regulation of PDGF merely is a manifestation of diabetic renal injury or whether PDGF plays an active role in the pathophysiology of diabetic nephropathy. The objectives of this study were to assess whether PDGF-dependent pathways are involved in the development of diabetic nephropathy and to determine the effects of PDGF receptor antagonism on this disorder and the processes associated with it.
**Materials and methods:** This study used the diabetic apolipoprotein E-knockout (apo E-KO) mouse, a model of accelerated diabetic nephropathy. Diabetes was induced by injection of streptozotocin in six-week old apo E-KO mice. Diabetic animals received treatment with a tyrosine kinase inhibitor which inhibits PDGF action, imatinib (STI-571, 10 mg/kg/day orally) or no treatment for 20 weeks. Non-diabetic apo E-KO mice served as controls.
**Results:** This model of accelerated renal disease with albuminuria as well as glomerular and tubulointerstitial injury was associated with increased renal expression of PDGF-B, proliferating cells and alpha-smooth muscle actin positive cells (Table 1). Furthermore, there was also increased renal transforming growth factor-beta1 (TGF-beta1) expression, increased accumulation of type I and type IV collagen as well as and macrophage infiltration. Imatinib treatment ameliorated both renal functional and structural parameters of diabetes as well as over-expression of growth factors, collagens, proliferating cells and a-smooth muscle actin positive cells as well as macrophage infiltration in the kidneys.
**Conclusion:** Tyrosine kinase inhibition with imatinib appears to retard the development of diabetic nephropathy.

Table 1. Indicators or renal injury and immunohistochemical studies.

| Parameters | Control (n=20) | Diabetes (n=20) | Diabetes + imatinib (n=14) |
|---|---|---|---|
| Urinary Albumin Excretion (μg/24 h) | $12.8 \pm 1.5$ | $49.6 \pm 3.6$*** | $28.9 \pm 2.9$*## |
| Glomerular Sclerosis Index | $1.418 \pm 0.162$ | $2.169 \pm 0.101$*** | $1.773 \pm 0.095$# |
| Tubulointerstitial area | $3.78 \pm 0.68$ | $6.78 \pm 0.59$* | $4.56 \pm 0.08$## |
| PDGF-B (%) | $0.035 \pm 0.015$ | $0.153 \pm 0.046$** | $0.037 \pm 0.016$## |
| Alpha-Smooth Muscle Actin (%) | $0.174 \pm 0.039$ | $1.243 \pm 0.091$*** | $0.122 \pm 0.008$### |
| Collagen I (%) | $0.020 \pm 0.004$ | $0.345 \pm 0.004$*** | $0.019 \pm 0.002$### |
| Collagen IV (%) | $0.063 \pm 0.005$ | $1.546 \pm 0.076$*** | $0.286 \pm 0.018$***### |
| TGF-beta in glomeruli (%) | $0.433 \pm 0.106$ | $4.079 \pm 0.309$*** | $1.249 \pm 0.041$***### |
| TGF-beta in tubules | $1.117 \pm 0.178$ | $2.467 \pm 0.250$*** | $1.356 \pm 0.170$### |
| Macrophages (F4/80,%) | $1.415 \pm 0.188$ | $2.881 \pm 0.272$*** | $0.935 \pm 0.095$### |
| Proliferating cells (Ki-67)/field | $1.30 \pm 0.14$ | $3.08 \pm 0.29$*** | $2.18 \pm 0.23$**## |

*, P<0.05, **, P<0.01, ***, P<0.001 vs. Control; #, P<0.05, ##, P<0.01, ###, P<0.001 vs. Diabetes.

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## 171

**Oxidative stress accounts for the resistance to the nitric oxide/cGMP/PKG pathway in aortic vascular smooth muscle cells from insulin resistant Zucker fa/fa rats: potential relevance in the pathogenesis of aterial hypertension in the insulin resistant states**

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**Background and aims:** We previously observed that arterial vascular smooth muscle cells (VSMC) from insulin-resistant (Zucker fa/fa) rats show, in comparison with those derived from insulin-sensitive (Zucker fa/+) rats, defects in the ability of nitric oxide (NO) to activate guanylate cyclase and therefore to increase the vasodilating cyclic nucletotide cyclic GMP (cGMP). Aim of the present study is to investigate whether these cells also present a resistance to the ability of cGMP to activate its specific kinase PKG and the putative role of oxidative stress in these abnormalities.
**Materials and methods:** In VSMC from Zucker fa/+ and Zucker fa/fa rats, obtained as primary cultures in our laboratory, we evaluated, both in the absence and in the presence of a 180-min preincubation with the anti-oxidant substances 300 U/ml superoxide dismutase (SOD)+250 U/ml catalase (n=5): i) intracellular concentrations of cGMP (RIA) in response to a 60-min incubation with 100 μM sodium nitroprusside (SNP), a NO donor; ii)

protein expression of the PKG substrate Vasodilator Stimulated Phosphoprotein (VASP) (Western Blot) in response to a 60-min incubation with the cGMP analog 8-bromo cGMP (500 µmol/l).
**Results:** i) baseline concentrations of cGMP (pmol/mg proteins) were higher in VSMC from Zucker fa/fa rats than in those from Zucker fa/+ rats (1.9 ± 0.1 and 0.83 ± 0.05, respectively, p=0.0001); ii) the cGMP increase in response to a 60-min incubation with SNP was smaller in VSMC from Zucker fa/fa rats (from 1.9 ± 0.1 to 2.1 ± 0.1 pmol/mg proteins, p=ns) than in VSMC from Zucker fa/+ rats (from 0.83 ± 0.05 to 3.42 ± 0.21 pmol/mg proteins, p=0.0001); iii) protein expression of phosphorylated VASP in response to 8-bromo cGMP (expressed in arbitrary units of the densitometric analysis of western blots) was lower in VSMC from Zucker fa/fa rats than in VSMC from Zucker fa/+ rats (12 ± 3.1 vs 25 ± 3.2, p=0.0001). In the presence of SOD+Catalase: i) baseline concentrations of cGMP were unmodified in Zucker fa/+ rats (0.83 ± 0.05 and 0.93 ± 0.15, ns) and significantly decreased in VSMC from Zucker fa/fa rats (from 1.9 ± 0.1 to 0.59 ± 0.09, p=0.0001); ii) a significant response of cGMP to SNP was restored in Zucker fa/fa rats (from 0.59 ± 0.9 to 1.8 ± 0.11 pmol/mg proteins, p=0.0001); iii)protein expression of phosphorylated VASP in response to 8-bromo-cGMP was not modified in Zucker fa/+ rats (25 ± 3.2 and 30 ± 4.1, ns) and significantly increased in Zucker fa/fa rats (from 12 ± 3.1 to 23 ± 2, p=0.04).
**Conclusions:** VSMC from the insulin-resistant Zucker fa/fa rats show, when compared to VSMC from insulin-sensitive Zucker fa/+rats: i) increased baseline concentrations of cGMP; ii) impaired cGMP response to NO; iii) impaired PKG response to cGMP. All these abnormalities are corrected by anti-oxidants and are therefore attributable to an increased oxidative stress. In conclusion, this study suggests the pivotal role of oxidative stress in the pathogenesis of arterial hypertension in the insulin resistant states.
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**Early abnormalities in retinal cell replication in a model that combines genetic hypertension and experimental diabetes mellitus**
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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 cell cycle and its regulators on neuro glial cells in retina in a model of genetic hypertension and experimental diabetes.
**Materials and methods:** Diabetes was induced in male 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 ≥15 mmol/L were included into the study and sacrified 15 days after the induction of DM. Cell proliferation was assessed by immunohystochemistry by the incorporation of 5-bromo-2'-deoxyuridine (BrdU), a thymidine analogue that incorporates into DNA in the *S* phase. The cell cycle inhibitors, $p27^{Kip1}$ and $p21^{Cip1}$ were evaluated by Western blot analysis and also by immunohystochemistry. The glial reaction was estimated by Western blot (WB) of glial fibrilar acidic protein (GFAP). Retinal capilary permeability was quantified by the Evans Blue method.
**Results:** The number of proliferating cell in the retina was significant higher in SHR (1.2 ± 1.00 cell/retinal section) than in WKY (0.15 ± 0.1, p<0.05). After 15 days of DM, there was a marked reduction in cell proliferation to 0,03 ± 0,06 cell/retinal section only in SHR rats (p=0.03). The expression of retinal $p27^{Kip1}$ estimated by WB, revealed a tendency of increasing expression in both diabetic groups (0.8 ± 0.4 vs 1.6 ± 0.8; 0.9 ± 0.4 vs 1.6 ± 0.4 densitometric units, p=0.09, respectively for WKY and SHR). The expression of $p27^{Kip1}$ among the retinal layers, revealed clear increasing of this protein in the ganglion cell layer of the retina in diabetic animals (5,8 ± 3,8 vs 78,3 ± 11,5 and 23,3 ± 11,5 vs 60,0 ± 14,1% of positivity for SHR and WKY respectively, p=0,0002). There was a marked blood-retinal barrier (BRB) breakdown in diabetic SHR (0.8 ± 0.6 vs 1.5 ± 0.4 µl plasma X *g* retinal dry $wt^{-1}$ X $h^{-1}$, p=0.04) not observed in WKY (1 ± 0.13 vs 0.9 ± 0.11). The glial reaction evaluated by GFAP expression and the expression of $p21^{Cip1}$ of did not differ among the studied animals (p=0.4).
**Conclusion:** The induction of diabetes in SHR rats promotes: decreasing in retina cell replication, increased cell cycle inhibitor ($p27^{Kip1}$) and BRB breakdown. These abnormalities not observed in diabetic normotensive rats may precede the early glial reaction in the diabetic retina and suggest a mechanism by which hyperglycemia and hypertension interacts in the development of DR.
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## 173

**Nitrergic-cholinergic neurodegeneration in cerebral arteries of streptozotocin-induced diabetic rats**
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**Background and aims:** Autonomic neuropathy has been shown to be an independent risk factor for stroke in diabetes. Parasympathetic nerves which are cholinergic and release nitric oxide (NO) are now known as „nitrergic nerves" and have been shown to innervate and relax cerebral arteries. It is not known whether these nerves are damaged during diabetes which might be an underlying cause for the decreased vasodilation and increased tone in cerebral arteries hence stroke. We therefore investigated the morphological changes to these nerves in an animal model of type-1 diabetes .
**Materials and methods:** Diabetes was induced in male Wistar rats with a single injection of streptozotocin (STZ; i.p.; 75 mg/kg). Some of the diabetic rats received insulin implants immediately or 8 or 12 weeks after STZ injection. Immunostaining using antibodies against neuronal nitric oxide synthase (nNOS), vesicular acetylcholine transferase (VAChT; cholinergic nerve marker), beta-tubulin, PGP9.5 (non-specific neuronal markers) and smooth muscle specific alpha-actin and TUNEL staining (to detect apoptosis) were performed in the cerebral arteries and pterygopalatine ganglia at the $8^{th}$, $12^{th}$, $16^{th}$ and $20^{th}$ weeks. nNOS was also measured using Western blotting.
**Results:** STZ injection resulted in hyperglycemia and arrest of weight gain. Insulin treatment corrected the blood glucose and body weight. The VAChT-positive cholinergic nerves which contain nNOS degenerated in two phases in the cerebral arteries of diabetic animals. In the first phase (the first12 weeks) nNOS decreased in the perivascular nitrergic axons but not in the cell bodies in the pterygopalatine ganglia. This phase was reversible with insulin treatment. In the second phase (after the $12^{th}$ week) nitrergic cell bodies in the ganglia went into apoptosis; this resulted in loss of nitrergic axons in the cerebral arteries. This degenerative phase was not reversible with insulin treatment. In both phases irreversible thickening of the smooth muscle layer of cerebral arteries was observed.
**Conclusion:** These results show for the first time nNOS depletion in the perivascular nitrergic nerves in the cerebral arteries of diabetic rats and suggest a biphasic model for nitrergic neurodegeneration which brings a new insight into the pathophysiology of stroke in diabetes.
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**Iron, diabetes and neurovascular dysfunction in rats**
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**Background and aims:** Oxidative stress plays an important role in the aetiology of the neural and vascular complications of diabetes. Transition metal homeostasis is disturbed by diabetes, contributing to the formation of damaging species such as hydroxyl radicals. The hypothesis to be tested is that if iron is important in this regard, then treatment with a selective iron chelator should protect against the development of diabetic neurovascular defects in rats. Furthermore, iron-loading of nondiabetic rats could mimic diabetic neurovascular dysfunction.
**Materials and methods:** The effects of 4 weeks of streptozotocin-diabetes with or without ferrous iron chelator treatment by 1,10-phenanthroline (50 mg/kg/day p.o.), and the effects of iron-loading (ferrocene, 100 mg/kg/day p.o.) of nondiabetic rats were examined. Measurements were made on nerve electrophysiology, sensory testing, and nerve perfusion by hydrogen clearance microelectrode polarography. Data are mean±SEM
**Results:** Diabetes caused 21.3 ± 1.1% (p<0.001) and 17.6 ± 1.0% (p<0.001) reductions in sciatic nerve motor and saphenous nerve sensory conduction velocity, respectively. Ferrous iron chelation with 1,10-phenanthroline was protective (motor, 99.8 ± 7.6%; sensory 82.9 ± 10.4%; p<0.001). Iron-loading of nondiabetic rats did not affect plasma glucose levels (6.3 ± 0.4 mmol/l c.f. 37.8 ± 3.3 mmol/l with diabetes). Nonetheless, iron-loaded nondiabetic rats had 17.5 ± 1.6% (p<0.001) motor and 12.7 ± 1.2% (p<0.001) sensory conduction deficits. Diabetes caused tactile allodynia, with a reduction in thresholds for foot withdrawal to electronic von Frey

hair analogue stimulation of 57.7 ± 3.8% ($p<0.001$). Thermal hyperalgesia was also apparent, with a 26.1 ± 4.9% reduction in the latency for foot withdrawal to noxious stimulation ($p<0.01$). Chelator treatment prevented the development of tactile allodynia and thermal hyperalgesia by approximately 96% ($p<0.01$). Iron-loading of nondiabetic rats also caused 73.0 ± 5.8% tactile allodynia ($p<0.001$) and 20.5 ± 5.4% ($p<0.01$) thermal hyperalgesia. Sciatic nerve and superior cervical ganglion blood flow were 44.2 ± 4.9% and 43.5 ± 2.9% reduced by diabetes ($p<0.001$), respectively. These defects were markedly attenuated (nerve, 94.8 ± 11.6%; ganglion, 89.9 ± 15.9%; both $p<0.001$) by chelator treatment. Iron-loading of nondiabetic rats produced deficits similar to those of diabetes, nerve and ganglion perfusion being 38.7 ± 4.1% and 43.3 ± 5.8% reduced ($p<0.001$), respectively. Histochemical staining for iron revealed an approximately 27% increase in staining intensity in sciatic epi/perineurium, which was particularly prominent in blood vessels, for both iron-loaded and diabetic groups ($p<0.05$). In the latter, this was largely prevented by chelator treatment ($p<0.05$).
**Conclusion:** The data show that iron-mediated processes play an important role in the development of diabetic neurovascular deficits in rats. Dysfunction was also noted in iron-loaded nondiabetic rats in the absence of hyperglycaemia. Impaired iron homeostasis may be a factor in explaining why it is difficult to prevent the chronic development of nerve and vascular complications in diabetes by good glycaemic control alone. Chelator treatment may have potential therapeutic relevance.
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# OP 30
# Insulin signalling cascade

## 175

**Insulin receptor autophosphorylation alone is not sufficient for signal transduction throughout the complete insulin signalling pathway**
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**Background and aims:** It is known from literature that small molecule insulin receptor agonists can mimic insulin action and therefore can be used as tools for studying insulin signaling. We therefore wanted to evaluate whether insulin-mimicing small molecules are able to trigger insulin-like responses on various levels of the insulin signaling cascade.
**Materials and methods:** Test compounds are applied *in vitro* to insulin receptor containing Chinese hamster ovarial cells (CHO-IR) or *in vivo* to female db/db mice. 2 hours after oral gavage of the compounds the animals were sacrificed and liver and muscle tissues were removed. Organ homogenates were analyzed by western blotting detection of the proteins with phosphosite-specific antibodies.
**Results:** We have identified two classes of insulin receptor kinase activators (IRKA-I and IRKA-II) which increase the autophosphorylation of the insulin receptor kinase *in vitro* in CHO-IR cells and after oral administration in muscle and liver tissue of db/db mice. Analysis with phosphosite-specific antibodies of lysates from CHO-IR cells after stimulation with compounds in theμM-range revealed a tyrosine phosphorylation pattern and a receptor kinase activity comparable to 1 nM insulin. However, in contrast to IRKA-I, IRKA-II failed to stimulate glucose uptake in this cell line. Combination experiments showed that small amounts of insulin and IRKA-II did not interfere with insulin signaling or insulin stimulated glucose uptake. Surprisingly, IRKA-II failed to elicit efficient IRS-1 tyrosine phosphorylation and consequently Akt-activation. In contrast, IRKA-I was able to stimulate receptor-dependent phosphorylation of both signaling molecules. A detailed analysis of intact cells by confocal laser scanning microscopy revealed a similar localization of insulin receptor phosphorylation signals after insulin, IRKA-I or IRKA-II stimulation.
**Conclusion:** Our data suggest that autophosphorylation of the insulin receptor alone is not sufficient to activate the complete insulin receptor signaling pathway down to the GLUT4 translocation machinery. Since it is know that insulin binding in addition to autophosphorylation triggers a conformational change and internalization of the receptor, it appears that IRKA-II is not able to mimic one or both features of insulin binding to its receptor. Therefore, we conclude that IR autophosphorylation can be mechanistically uncoupled from downstream signaling events and that additional activation steps are required to transduce the insulin signal throughout the complete signaling pathway.

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**Serine phosphorylation of IRS-1 by protein kinase C modulates insulin signal transduction**
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**Background and aims:** Recent studies have focused on Ser/Thr phosphorylation of the IRS proteins as a key negative-feedback control mechanism of insulin signaling. This modification uncouples the IRS proteins from their upstream and downstream effectors and down-regulates signal transduction in response to insulin, under physiological and pathophysiological conditions. AIMS: 1) To analyze the influence of different metabolic stimuli on our recently identified protein kinase C phosphorylation site at Ser-318 of IRS-1 in transient transfected cell lines, in non-transfected tissue-specific cells and in various metabolic relevant tissues; and 2) to investigate signaling events affected by pSer-318 under these conditions.
**Materials and methods:** We detected the phosphorylation of Ser-318 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 pSer-318 have been studied using a Ser-318 to Ala mutant of IRS-1.
**Results:** We observed an increase in the phosphorylation of Ser-318 under various stimuli (e. g. insulin), which were accompanied by a decreased Tyr phosphorylation of IRS-1. Stimulation of hepatoma cells with 100 nM

insulin for 6 h resulted in a peak of Ser-318 phosphorylation after 60 min which decreased after 2 h to the basal level and remained there until the end of the experiments. The pTyr of IRS-1 showed a temporal inverse correlation, i. e. decrease within 60 min and increase after 2 h back to the most intense level, which was achieved after 5 min of insulin stimulation. This effect appears to be specific for IRS-1 since IRS-2 tyrosine phosphorylation is not concomitantly decreased. Furthermore, investigating the downstream signaling events influenced by pSer-318 in BHK-IR cells, we observed a decreased insulin receptor/IRS-1 interaction and a reduced tyrosine phosphorylation of IRS-1, as well as a negative influence on the PKB/Akt- and the MAPK-pathway demonstrated as a decreased phosphorylation of Thr-308 of PKB/Akt and of Thr-202/Tyr-204 of ERK2. *In vivo* studies in various tissues also showed the phosphorylation of Ser-318 in IRS-1 under different metabolic stimuli.
**Conclusion:** These results suggest that the phosphorylation of Ser-318 contribute to the PKC-induced inhibitory effect of metabolic stimuli (e. g. hyperinsulinemia) on insulin signal transduction.
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**Shp2 mediates phorbol ester-stimulated phosphorylation of serine 307 in insulin receptor substrate 1**
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**Background and aims:** The detailed molecular mechanisms that cause insulin resistance are still not fully understood. In recent years, protein kinase C (PKC) and protein tyrosine phosphatases, among them the cytoplasmic Src homology 2 domain-containing protein tyrosine phosphatase 2 (Shp2), have been shown to negatively regulate insulin-dependent pathways. Since expression of Shp2 in mouse embryonic fibroblasts (MEF) induced a shift of insulin receptor substrate 1 (IRS-1) in the SDS-polyacrylamide gel, we analysed whether this shift was due to serine phosphorylation of IRS-1. In particular, we looked at serine 307 of IRS-1, since this phosphorylation site is reported to play a role in insulin resistance.
**Materials and methods:** MEF cells were infected with retroviruses encoding human wild-type Shp2, the double mutant Shp2-S576/591A and a phosphatase-negative Shp2. Stimulated MEF cells were lysed, and cell protein was separated by SDS-polyacrylamide gel electrophoresis, transferred to a nitrocellulose membrane, and processed for immunodetection with the appropriate antibodies. Bound antibodies were visualized using horseradish peroxidase-conjugated antibodies and the ECL system.
**Results:** In MEF cells expressing wild-type Shp2, 12-O-tetradecanoylphorbol 13-acetate (TPA)-induced activation of PKC strongly increased phosphorylation of serine 307 of IRS-1. This phosphorylation was blunted in vector-transfected Shp2 knockout cells. Shp2 is, as previously shown, phosphorylated on serine residues 576 and 591 by PKC. However, in cells expressing the mutant Shp2-S576,591A or a phosphatase-negative Shp2 serine 307 phosphorylation was not impaired. Preincubation with the PKC inhibitor bisindolylmaleimide I, with the c-jun N-terminal kinase (JNK) inhibitor SP600125 or with the phosphatidylinositol 3 (PI 3)-kinase inhibitor wortmannin blocked TPA-stimulated serine 307 phosphorylation. Anisomycin stimulated serine 307 phosphorylation independently of Shp2.
**Conclusion:** In conclusion, PKC-dependent phosphorylation of serine 307 of IRS-1 is mediated by Shp2, PI 3-kinase and JNK with JNK lying downstream of Shp2 and PI 3-kinase. Furthermore, we found that serine residues 576 and 591 as well as the phosphatase activity of Shp2 are not necessary for this regulation.

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**The p66Shc protein controls glucose transport responses in skeletal muscle cells through Erk-dependent regulation of the actin cytoskeleton**
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**Background and aims:** Intracellular signaling by growth factor tyrosine kinase receptors involves tyrosine phosphorylation and activation of the Shc proteins. While p52Shc and p46Shc mediate cell growth and transformation, the p66Shc protein isoform has recently been shown to possess distinct signaling properties and is activated under conditions of cellular stress. The objective of this study was to define the role of p66Shc in glucose transport responses of skeletal muscle cells.
**Materials and methods:** L6 skeletal muscle cells with marked and persistent reduction of the p66Shc protein (L6/Shcas) were generated by stable transfection of specific antisense oligonucleotides.
**Results:** In control L6 myoblasts, IGF-I stimulation resulted in a 2-fold increase in glucose transport, measured by determining the rates of [3H]2-deoxy-D-glucose uptake ($p<0.05$ vs. basal). By contrast, L6/Shcas myoblasts showed dysregulation of the glucose transport system, with 10-fold increase in basal transport and no response to IGF-I stimulation ($p= 0.55$ vs. basal). The analysis of glucose transporter protein content by immunoblotting of total cellular membranes with specific antibodies revealed 6-fold and 4-fold increases in GLUT1 and GLUT3 protein levels, respectively, in L6/Shcas compared to control myoblasts, whereas GLUT4 protein content was unchanged. The reduction of p66Shc also caused total disruption of the actin cytoskeleton, visualized by immunofluorescence with rhodamine-conjugated falloidin, and loss of the normal skeletal muscle cell phenotype with altered IGF-I-induced mitogenesis and differentiation. Furthermore, the reduction of p66Shc in skeletal muscle cells was associated with constitutive activation of the intracellular kinases MEK and Erk-1/2 (respectively 5-fold and 3-fold higher than control, $p<0.05$). However, pretreatment of L6/Shcas cells with the selective MEK inhibitor PD98059 completely restored the actin cytoskeleton and cellular phenotype, and corrected the impairment in glucose transport with reduction of the elevated basal transport and reappearance of the IGF-I effect.
**Conclusion:** The reduction of p66Shc in L6 skeletal muscle cells results in increased expression of GLUT1 and GLUT3 and dysregulation of the glucose transport system that becomes unresponsive to IGF-I. These effects occur through constitutive activation of MEK/Erk and depolimerization of the actin cytoskeleton. Therefore, the p66Shc protein controls the MEK/Erk pathway and the actin cytoskeleton turnover, which are both essential for normal metabolic responses in skeletal muscle cells.

## 179

**Phosphatidylinositol-3-phosphate, a new crucial player in insulin signalling, is generated via a TC10-mediated phosphoinositide 3-kinase C2alpha activation**
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**Background and aims:** Disposal of glucose into fat and muscle cells requires the insulin-induced translocation of the GLUT4 glucose transporter protein from intracellular storage sites to the plasma membrane. GLUT4 translocation occurs through activation of at least two distinct pathways, involving phosphoinositide 3-kinase (PI 3-K) and the small GTP-binding protein TC10 respectively. We have recently identified phosphatidylinositol-3-phosphate (PtdIns-3-P) as a new important component in the TC10-dependent pathway, involved in GLUT4 translocation. We have reported that insulin generates a pool of PtdIns-3-P at the level of the lipid rafts subdomain of the plasma membrane. This formation requires the insulin-dependent activation of TC10 and is relatively resistant to PI 3-K inhibitors. These data strongly suggest that the enzyme involved in this process might be the a_isoform of class II PI 3-K (PI 3-KC2a) that is wortmannin resistant and is activated by insulin. The aim of this work was to determine the enzyme responsible for generation of the insulin-dependent pool of PtdIns-3-P.
**Materials and methods:** Intracellular localization of different proteins was assessed by confocal microscopy and subcellular fractionation studies. PI 3-KC2a activity was assessed by in vitro lipid kinase assays.
**Results:** Insulin translocates the endogenous enzyme PI 3-KC2a to the plasma membrane of L6 cells. Translocation is rapid, being already visible at 1.5 min of stimulation, and transient, disappearing after 15 min of stimulation. This is consistent with the rapid and transient formation of PtdIns-3-P that we have reported. Pre-treatment with cholesterol-extracting drugs inhibits the insulin-mediated translocation of PI 3-KC2a indicating that preservation of intact lipid rafts subdomain is necessary for this process. Overexpression of a constitutively active TC10 mutant per se activates PI 3-KC2a as assessed in in vitro kinase assays whereas overexpression of a dominant negative mutant completely inhibits the insulin-dependent PI 3-KC2a activation. In addition the isolated C-terminal C2 domain of PI 3-KC2a fused to the GFP translocates to the plasma membrane upon insulin stimulation suggesting that this domain is responsible for PI 3-KC2a membrane targeting.
**Conclusion:** Our previous work has clearly identified PtdIns-3-P as a new component in the TC10-dependent insulin cascade that plays a crucial role in GLUT4 translocation. Data presented here indicate that this new second messenger is generated through an insulin/TC10-mediated activation of the enzyme PI 3-KC2a. These data clearly define for the first time the role of PI 3-KC2a in insulin signalling. Furthermore these data indicate that insulin not only can activate PI 3-KC2a but it can also regulate PI 3-KC2a intracellular localization, possibly through its C2 domain. This is the first evidence that the intracellular localization of a class II PI 3-K can be regulated by agonist stimulation. This is of particular interest in the study of

diabetes since it may represents a novel mechanism of regulation of insulin signalling.
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**Blockade of phosphoinositide 3-kinase c2*a* gene expression suppresses insulin stimulation of ERK activity but not of Akt activity and induces apoptotic cell death in CHO-IR cells**

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**Background and aims:** Phosphoinositide 3-kinase (PI3 kinase) is an important signaling molecule to execute diverse roles upon insulin-receptor binding. It is known that several distinct classes of PI3 kinase exist and the best understood of these is the class IA PI3 kinase. Recently, different isoforms ($\alpha, \beta, \gamma$) of class II PI3 kinase have been identified and PI3 kinase c2$\alpha$ isoform has known to be stimulated by insulin, however, its function to stimulate downstream signaling cascades has been not understood. The present study aimed to investigate the specific role of PI3 kinase c2$\alpha$ isoform in stimulating protein kinase B/Akt or extracellular signal-regulated protein kinase (ERK) by insulin in chinese hamster ovary cells expressing human insulin receptor molecules (CHO-IR cells). It was also investigated whether PI3 kinase c2$\alpha$ isoform can play a critical role in maintaining cellular viability.
**Materials and methods:** To suppress gene expression of PI3 kinase c2$\alpha$ isoform within cells, antisense oligonucleotides were introduced with liposome-mediated transfection methods.
**Results:** Transfection of CHO-IR cells with antisense oligonucleotides decreased the intracellular protein content of PI3 kinase c2$\alpha$ isoform, resulted in the suppression of ERK activity but not of Akt activity which were stimulated by insulin. Moreover, such a blockade of PI3 kinase c2$\alpha$ expression induced a wide variety of apoptotic cell death phenomena including internucleosomal cleavage of DNA (laddering), nuclear chromatin condensation and accumulation of subdiploid (<2N) cells from flow-cytometric analysis and others.
**Conclusion:** These results suggest that PI3 kinase c2$\alpha$ isoform is a novel signaling molecule to specifically mediate insulin stimulation of ERK activity when necessary. In addition, PI3 kinase c2$\alpha$ isoform might also play role(s) to maintain cellular viability or to promote cellular proliferation.

# OP 31
# New oral agents

## 181

**Muraglitazar, a novel non-TZD dual PPAR-alpha/gamma agonist, increases UCP-1 expression and decreases the size of adipocytes in white adipose tissue and enhances the basal metabolic rate in fat-diet fed hamsters.**

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**Background and aim:** Muraglitazar (BMS-298585) is a non-TZD, oxybenzylglycine dual PPAR-alpha/gamma agonist in clinical development for the treatment of type 2 diabetes and its associated dyslipidemia. Previously, muraglitazar has been demonstrated to improve glucose and lipid profiles in various diabetic and hyperlipidemic animal models. In addition, muraglitazar has been shown to diminish body weight gain and to reduce inguinal fat depot size in fat diet-fed hamsters. In order to understand the mechanism(s) of the effects of muraglitazar on body weight gain and inguinal fat depot size, we have investigated its effect on: 1) uncoupling protein (UCP) gene isoform expression in inguinal white adipose tissue (WAT), 2) the adipocyte cell size of inguinal WAT, and 3) resting O2 consumption in fat-diet fed hamsters.
**Materials and methods:** Fat-diet fed Golden Syrian hamsters, which were treated with muraglitazar (30 mpk p.o for 45 days), were evaluated in metabolic chambers for basal O2 consumption and their inguinal WAT samples were analyzed for adipocyte cell size and UCP gene expression.
**Results:** PCR analysis of WAT RNA from muraglitazar-treated hamsters showed significant induction of UCP-1 gene expression (10.1-fold induction) but not UCP-2 or UCP-3 gene expression. Histochemical analysis of WAT samples with UCP-1 antibody confirmed the PCR data. Histological analysis of WAT samples from muraglitazar-treated hamsters showed a decrease in the average size of adipocytes (60% increase in total cell count per tested area). Finally, metabolic analysis of muraglitazar-treated hamsters showed a higher rate of basal O2 consumption (14% increase) relative to control animals.
**Conclusion:** Muraglitazar treatment of fat diet-fed hamsters stimulates UCP-1 gene expression and decreases the average size of adipocytes in inguinal WAT. These changes are likely mediated through the PPAR-gamma agonist activity of muraglitazar, which enhances pre-adipocyte differentiation, promotes apoptosis of large mature adipocytes and stimulates the transcription of UCP-1 gene. The increase in basal metabolic rate (as evidenced by higher rate of O2 consumption) is likely due to the elevated UCP-1 expression, and results in reduced inguinal fat depot size and diminished body weight gain in muraglitazar treated animals. The smaller-sized adipocytes, which are considered metabolically more active and insulin sensitive, may contribute to the improvement in glycemic control and lipid profiles observed in muraglitazar treated animals.

## 182

**Long-term efficacy of the DPP-4 inhibitor, LAF237, in patients with type 2 diabetes inadequately treated with metformin**

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**Background and aims:** Although most oral antidiabetic agents are initially effective at achieving acceptable glycemic control in patients with type 2 diabetes (T2DM), it is well-known that $HbA_{1c}$ levels increase as the disease progresses and good glycemic control is seldom maintained in the long-term, even with combination therapy. LAF237 is an agent that potentiates the effects of the incretin hormones, GLP-1 and GIP, by inhibiting the enzyme (DPP-4) responsible for their degradation. We previously reported that 12-wk treatment with LAF237 (50 mg, qd) reduced $HbA_{1c}$ by 0.6% in patients with T2DM continuing their ongoing stable dosage of metformin (MET, 1500 to 3000 mg/day). Here we relate findings from a 40-wk extension of that study.
**Materials and methods:** Forty-two patients (62% male) received LAF237 plus MET (LAF/ MET) and 29 patients (76% male) received placebo plus

MET (PBO/MET) and $HbA_{1c}$ was measured periodically for 52 weeks and all adverse events (AEs) were recorded.
**Results:** At baseline (BL) the mean age, BMI and $HbA_{1c}$ of participants were 56.7 y, 29.7 kg/m$^2$ and 7.7%, respectively, and did not differ significantly between treatment groups. Table 1 shows $HbA_{1c}$ (%) during the 52-wk treatment (Mean ± SEM, ITT population).

| Treatment (n) | Baseline | Wk 12 | Wk 16 | Wk 24 | Wk 36 | Wk 52 |
|---|---|---|---|---|---|---|
| LAF/MET (42) | 7.6 ± 0.1 (42) | 7.1 ± 0.1 (42) | 7.1 ± 0.1 (42) | 7.1 ± 0.1 (40) | 7.1 ± 0.1 (36) | 7.1 ± 0.1 (33) |
| PBO/MET (29) | 7.8 ± 0.1 (29) | 7.8 ± 0.1 (29) | 7.8 ± 0.1 (28) | 7.9 ± 0.1 (29) | 8.0 ± 0.2 (28) | 8.3 ± 0.2 (26) |

In patients taking PBO/MET, $HbA_{1c}$ increased from Wk 12 to Wk 52 at a rate of 0.0656%/mo, whereas the rate of $\Delta HbA_{1c}$ in patients taking LAF/MET (0.0128%/mo) was less than that in patients taking PBO/MET ($P<0.05$) and not significantly different from zero. The between-group difference in adjusted mean $\Delta HbA_{1c}$ and ΔFPG at endpoint were −1.1 ± 0.2%, ($P<0.0001$) and −1.1 ± 0.5 mmol/l ($P < 0.05$), respectively. Further, 41.7% of patients taking LAF/MET with a BL $HbA_{1c}$ ≥7.0% achieved an endpoint $HbA_{1c}$ < 7.0%, *vs* 10.7% of patients taking PBO/MET. Treatment was generally well-tolerated: 76.2% and 89.7% of patients taking LAF/MET and PBO/MET, respectively, completed the study, with 2 patients in each group discontinuing due to an unsatisfactory therapeutic effect. The most common AE was nasopharyngitis, occurring in 14% of patients in both groups. Hypertension and hypoglycemia were observed in 3 patients taking LAF/MET (7.1%) and no patient taking PBO/MET. No incident of hypertension was suspected to be study drug-related, all hypoglycemic episodes were considered mild and one was suspected to be drug-related. Body weight decreased by 0.2 kg in both groups.
**Conclusion:** These findings attest to the durability of efficacy and tolerability of LAF237 in MET-treated patients with T2DM and suggest that this DPP-4 inhibitor may modify progression of the disease.

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**Liver type glucokinase can be activated by LXR**
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The regulation of hepatic glucose metabolism is important in glucose homeostasis and liver glucokinase plays central role in this process. It was known that hepatic glucokinase expression is known to be regulated by insulin and GK expression was also induced by LXR agonist in livers. Because LXRα mediate certain actions of insulin on gene expression, gene regulation by LXRα is particularly interesting in primary insulin target tissue such as liver and adipose tissue. Liver X receptor (LXR) is known to play an important role in the regulation of lipid, cholesterol and carbohydrate metabolism. However, its role in the glucose metabolism is not well understood. Currently, it is reported that LXR agonist, T0901317, improves glucose tolerance in the rat. Thus, it is possible that glucokinase, one of the key enzymes in the glycolysis, could be a target of LXR action.
In this study, we have dissected the promoter region of rat liver glucokinase promoter and localized the *cis*-element binding LXR (LXRE). The LXRE is supposed to be localized in the -109/-80 and -59/-25 region of the promoter. Other data suggest that the region is responsible for direct binding of LXR and induction of the transcription of glucokinase. Also, LXR ligand induced the transcription of glucokinase in primary rat hepatocytes. From these results, it is assumed that LXR improves glucose tolerance by activating glucokinase in liver.
We show that the glucokinase promoter is direct transcriptional target for the LXR/RXR heterodimer. These data support that LXR agonists can be used as anti-hyperlipidemic as well as anti-hyperglycemic drugs for reducing blood glucose level in type 2 diabetic patients.

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**Characterization of the mechanism of action and antidiabetic activity of MB06322, a potent and selective inhibitor of fructose 1,6-bisphosphatase**
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**Background and aims:** MB06322 is an oral prodrug of a novel agent (MB05032) designed to inhibit specifically fructose 1,6-bisphosphatase (FBPase), a key enzyme of gluconeogenesis (GNG). Upregulated endogenous glucose production (EGP) is a common finding in type 2 diabetes (T2DM). It contributes significantly to postprandial and fasting hyperglycemia and has been attributed to increased GNG. The aim of the studies was to determine the potential of MB06322 as an antidiabetic agent through characterization in relevant *in vitro*, cellular, and *in vivo* models, including the Zucker Diabetic Fatty (ZDF) rat.
**Materials and methods:** Inhibition of recombinant human liver FBPase was assessed via an enzyme-coupled colorimetric assay. Hepatocytes were isolated using a standard collagenase perfusion method and compounds tested in fresh, suspension culture format. In rodent studies, glucose was determined in blood samples obtained from the tail vein. Liver samples were freeze-clamped under anesthesia for the enzymatic determination of GNG pathway intermediates. Rates of EGP were determined using standard $^3$H-6-glucose tracer techniques. The contribution of GNG to EGP was assessed by means of the deuterated water method.
**Results:** MB05032 inhibited human liver FBPase ($IC_{50}$ = 20 nM) synergistically with a natural effector of the enzyme, fructose 2,6-bisphosphate. In normal rat, ZDF rat, or human hepatocytes, MB06322 inhibited glucose production from common GNG substrates more potently than metformin (($IC_{50}$ 0.2–2 μM vs. > 2 mM, respectively). Infusion of MB05032 (1 mg/kg/h) to fasted, normal rats via the tail vein resulted in 50% inhibition of EGP,and a progressive lowering of blood glucose (BG). In the ZDF rat, oral MB06322 elicited the expected changes in hepatic GNG precursor/intermediate levels, dose-dependent inhibition of glucose production from $^{14}$C-bicarbonate (at ≥10 mg/kg), inhibition of EGP (at ≥30 mg/kg) and a reduction in the fractional contribution of GNG to EGP (63% at maximal dose). BG lowering was observed both in fasting and freely-feeding, mature ZDF rats, without incidence of hypoglycemia. In young ZDF rats, chronic treatment (0.4% food admixture, 6 weeks) prevented the development of hyperglycemia and associated symptoms of diabetes such as polydipsia and glycosuria. The combination of MB06322 with glyburide (acute OGT model) or with insulin sensitizers such as troglitazone (3-4 week treatment) resulted in significantly improved antidiabetic activity in the ZDF rat relative to any of the therapies on their own.
**Conclusion:** The findings indicate that (1) MB05032 is a potent allosteric inhibitor of FBPase, (2) MB06322, a prodrug of MB05032, lowers blood glucose *in vivo* specifically via the inhibition of GNG (3) GNG is a major contributor to fasting and postprandial hyperglycemia, and (4) MB06322 either alone or in combination with approved agents, may afford a new treatment for the prevention or treatment of T2DM.

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**Glucose lowering and antidiabetic activity of a potent and selective inhibitor of fructose 1,6-bisphosphatase (MB06322) in normal and diabetic rodents**
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**Background and aims:** Upregulated endogenous glucose production is a common finding in Type 2 diabetes (T2DM). It contributes significantly to postprandial and fasting hyperglycemia and has been linked to increased gluconeogenesis (GNG). MB06322 is a novel, oral prodrug of a potent and selective inhibitor (MB05032) of a rate-limiting enzyme of GNG, fructose 1,6-bisphosphatase (FBPase). The primary purpose of the studies described was to assess the glucose lowering potential of MB06322 in isolated rat hepatocytes, in fed and fasted normal rats, and in diabetic mice and rats. A secondary purpose was to compare the activity of MB06322 to that of metformin, a commonly prescribed drug reported to act in part via GNG inhibition.

**Materials and methods:** Hepatocytes were isolated from fasted Sprague-Dawley (SD) or fed Zucker Diabetic Fatty (ZDF) rats by standard collagenase perfusion methods and treated with test compounds in fresh suspension culture format. ZDF rats or db/db mice were gavaged with test compounds in the fed or overnight-fasted state. In some studies drug was administered as a food admixture. Blood glucose (BG) was measured in samples obtained from the tail vein.
**Results:** In hepatocytes isolated from fasted SD rats or fed ZDF rats, MB06322 blocked glucose production from lactate (LAC)/pyruvate, alanine or glycerol with an $IC_{50}$ of ~2 μM whereas metformin was weakly active ($IC_{50}$ >2 mM). Significant antihyperglycemic activity (ΔBG 7%250 mg/dL) was demonstrated both in 6-h fasted and fed ZDF rats (1%13 weeks old) treated with MB06322 (30 mg/kg and above). Modest blood LAC elevation was observed in these studies. Glucose lowering in the fed ZDF rat following acute administration of metformin (300 mg/kg) was associated with similar LAC elevation. Interestingly, in the ZDF rat MB06322 but not metformin blocked the incorporation of $^{14}C$-bicarbonate into glucose in a dose-dependent manner. In the db/db mouse, MB06322 lowered BG (ΔBG 100 mg/dL at 30 mg/kg) without altering LAC or triglycerides (TG). MB06322 administration to 6-week old ZDF rats for 6 weeks (0.4% food admixture) fully prevented the development of hyperglycemia, polydipsia and glycosuria in 5 of 8 treated animals without incidence of hypoglycemia, and attenuated hyperglycemia in the remaining 3. LAC was not elevated in the animals in which hyperglycemia was prevented, nor was TG homeostasis significantly altered. Furthermore, oral glucose tolerance and the insulin secretory response were markedly improved. Metformin (0.4% food admixture) raised blood lactate but showed marginal antidiabetic activity in this chronic setting.
**Conclusion:** MB06322 is a potent inhibitor of glucose production from all common GNG substrates in hepatocytes, and an effective glucose lowering agent in fasted normal and diabetic rodents. Importantly, MB06322 was ~1000-fold more potent than metformin in isolated normal and diabetic hepatocytes and unlike metformin, prevented the development of hyperglycemia and associated metabolic dysfunction in the ZDF rat. The antidiabetic activity of MB06322 underscores the key role of GNG and glucose exposure in the manifestations of the disease. Overall, the acute and chronic *in vivo* data suggest that MB06322 may provide a novel therapy for the treatment of early as well as established T2DM.

## 186

### Rimonabant improves glycemic control parameters and glucose tolerance in overweight/obese non diabetic subjects with dyslipidemia. RIO-Lipids trial

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**Background and aims:** Rimonabant is the first selective cannabinoid type 1 ($CB_1$) blocker developed for the treatment of obesity and smoking cessation. Preclinical studies demonstrated the role of the endocannabinoid system, via the $CB_1$ receptor, in the central and peripheral regulation of energy balance, as well as in the control of nicotine dependence. Furthermore, Phase 2 studies established the efficacy of rimonabant in obesity and smoking cessation.
RIO-Lipids is the first of four Phase 3 studies conducted in a total of over 6,000 overweight/obese subjects investigating the drug's effectiveness on body weight and metabolic risk factors including a fasting glucose (FG) and insulin (FI) and OGTT calculating AUC.
**Materials and methods:** RIO-Lipids is an international, multicenter, double-blind, placebo-controlled, one year treatment study. Main inclusion criteria were 27 < BMI ≤ 40 kg/m$^2$, untreated dyslipidemia with 1.50 g/L (1.69 mmol/L) ≤ TG ≤ 7g/L (7.9 mmol/L) and/or TC/HDL-C > 4.5 (female), > 5 (male) with FG < 1.26 g/L (< 6.99 mmol/L).
1,036 subjects, 407 men and 629 women, mean age 47.8 years, mean BMI 34.0 kg/m$^2$, mean body weight (BW) 96.1 kg, mean waist circumference 107.1 cm, were randomized to receive placebo, rimonabant 5 mg or rimonabant 20 mg once daily with a mild hypocaloric diet. Primary efficacy end points were weight loss and weight maintenance at 1 year. OGTT with 0', 30', 60' and 120' time point measurements was performed at baseline, 6 months and 12 months for glucose and insulin levels
**Results:** A reduction in BW was seen in the 3 groups 6.9 (6.1)Kg, 3.1(4.8)Kg and 1.5 (5.0)Kg for rimonabant 20 mg, rimonabant 5 mg and placebo respectively. The differences between rimonabant 20 mg and rimonabant 5 mg over placebo were highly significative p<0.001)
Treatment with rimonabant 20 mg for one year significantly reduced the percentage of subjects fulfilling the NCEP ATPIII clinical criteria for the metabolic syndrome compared to placebo (52.9% , 55.9% and 51.9% at baseline versus 25.8%, 40%, and 41% after one year treatment for rimonabant 20 mg, rimonabant 5 mg and placebo respectively, p<0.0001 for rimonabant 20 mg over placebo). Rimonabant 20 mg significantly decreased TG and increased HDL-C ( p< 0.001). Fasting insulin increased by 0.9 micro IU/ml in the placebo group and decreased by 1.7 micro IU/ml in the 20 mg group (p=0.016). The overall plasma glucose and insulin responses to the 75 g oral glucose load were significantly improved with rimonabant 20 mg versus placebo (mean AUC (SEM) change for glucose= -46(13) mmol/L*min- p<0.001 and for insulin levels = -2171(346) μIU/mL*min - p<0.001). Adiponectin levels significantly improved with rimonabant at 20 mg versus placebo (mean adiponectin change=1.6 μg/mL -p<0.001) which represents a 41% increase between baseline and 1 year in the rimonabant 20 mg group.
**Conclusion:** Rimonabant, a novel CB1 blocker induced marked weight loss and improves glycemic control in overweight/obese patients with dyslipidemia. The OGTT results are compatible with an improved insulin sensitivity in this population and are consistent with the effects seen on adiponectin.
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# OP 32

# Audit and strategies to improve diabetes care

## 187

**Primary care diabetes case management: an innovative strategy for overcoming barriers and enhancing diabetes outcomes**

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**Background and aims:** General Practitioners (GPs) are challenged with an aging population and managing many patients with multiple chronic illnesses. GPs communicate that despite available knowledge and tools, the assessment and management of the many components of diabetes is complex and time-consuming. They identify, that they need the assistance of expert diabetes health care professionals, at the primary care level, in implementing diabetes best practices. In response to this identified need, tertiary care diabetes nurse case managers partnered with community GPs to assist them in prevention and optimal management of patients at risk and challenged with Type 2 DM.
**Materials and methods:** In May 2003, a pilot project known as Primary Care Diabetes Case Management was launched. This initiative involved a partnership between 2 busy primary care offices (1 urban; 1 rural) and the Endocrinology Division of a large tertiary organization. A model was designed and goals identified were, collaborative knowledge transfer and enhanced metabolic control. The initiative was designed such that 2 tertiary nurse case managers with specialties in diabetes, would work 5 hrs per day, 1 day a month. Employing the principles of case management, these nurses were involved in clinical care, tracking clinical outcomes and facilitating process redesign opportunities. The nurses independently interviewed and assessed 7 patients per half-day (40 minutes per new patient and 20 minutes for return visits). In a five-minute review with the GP at the end of each patient visit the nurse updated the physician regarding the patients status, and discussions were had regarding the nurses management recommendations. Patients were followed on average for 6 months based on the nurses assessment of patients glycemic control and needed support. The pilot was originally intended to run for 8 months, however, based on successful outcomes, the end date was extended indefinitely and not only continues to run in the original venues but the strategy is now being assessed for provincial expansion.
**Results:** At 11 months, the teams have worked collaboratively with 80 patients. Patients, nurses and physicians positively indicate this initiative has enhanced care. Random chart audits completed 6 months into the program noted 85% of patients achieved a decrease in their A1 c. Of these, greater than 75% achieved target A1 c levels < 7% within the first 3 months. Other clinical findings were significant increases in appropriate management of dyslipidemia. Greater than 50% of patients had a dyslipidemia agent initiated or increased during this time frame. Multiple patients previously resistant to medication management strategies have agreed to move from lifestyle management to medication management. Patients communicate this change in attitude is a result of clear, timely education and a greater understanding of the disease and risks of uncontrolled diabetes. From a process redesign perspective, the nurses have organized the storage of diabetes data in GP charts using a 1-page comprehensive flow sheet/assessment tool. The nurses have also facilitated timely links to other diabetes healthcare professionals as needed.
**Conclusion:** This innovative healthcare delivery strategy linking tertiary and primary diabetes care professionals, through a diabetes nurse case manager, has optimized GP time management, and facilitated evidence based diabetes best practice, resulting in enhanced diabetes outcomes and knowledge transfer.
*The authors thank GlaxoSmithKline for their unrestricted support of this project.*

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**Diabetes specialist nurse-led intervention to treat and control glycaemic regulation, hypertension and hyperlipidemia in patients with diabetes mellitus Type 2: a randomised controlled trial**

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**Background and aims:** In recent years the Dutch health care system is struggling to meet the growing number and the increasing needs of patients with diabetes and the lack of medical doctors. Our objective is to compare diabetes control, including hypertension and hyperlipidemia, in patients referred to a diabetes outpatient clinic receiving medical care provided by a diabetes specialised nurse (DSN) with patients receiving usual care by a diabetologist.
**Materials and methods:** In this 12-month prospective, open, randomised, controlled multicenter-trial, 50 patients with type 2 diabetes mellitus referred to three hospitals in the Netherlands, were randomly assigned to the control group (usual care by a diabetologist) or to the intervention group (diabetes care, including treatment and control of hypertension and hyperlipidemia, given by DSN following detailed protocols and algorithms). Primary outcome measures were HbA1 c, blood pressure (BP) and lipid profile. Secondary outcome measures were subjects' diabetes-related quality of life, satisfaction with treatment and total medical costs. Measurements were done by an independent medical doctor at baseline, after 6 and 12 months.
**Results:** 47 patients completed the study. Except for total cholesterol, which was significantly higher in the control group (5.6 vs. 4.8), no differences were found in baseline characteristics. After 12 months mean HbA1 c in the intervention group decreased from 9.0 (SD 1.4) to 7.3 (SD 0.7) and in the control group from 8.6 (SD 1.4) to 7.6 (SD 1.1). Compared with the control group, HbA1 c decreased 0.8% more (95% confidence interval [CI]: 0.05% to 1.6%) in the intervention group (p=0.032). Between the treatment groups, BP, diastolic BP, total cholesterol and cholesterol/HDL ratio were not significantly different at the end of the study.
After one year the number of patients who met the treatment goals were comparable for both groups (DSN vs. diabetologist): HbA1 c < 8.5% (96% vs. 83%), blood pressure < 150/85 mmHg (22% vs. 22%), total cholesterol < 5 mmol/l (70% vs. 72%), cholesterol/HDL ratio < 4 (78% vs. 78%).
**Conclusion:** In a secondary health care situation in which patients were randomly assigned to either a DSN or a diabetologists, glycaemic control was better in patients assigned to the nurse and other outcomes were comparable. To combat increasing waiting lists, a professional with less training and less experience may be able to fulfil the role of the medical doctor in the treatment of type 2 diabetes patients in a secondary health care system.

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**Evidence-Based Health Care (EBHC) for diabetes educators - knowledge gain in different course formats**

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**Background:** The recent paradigm shift in therapeutic counselling towards informed shared decision making (ISDM) requires new skills and competences of the therapeutic team. In chronic diseases such as diabetes with its multifaceted interventions ISDM may be particularly valuable. Diabetes educators already play a decisive role in the information process of patients with diabetes and as intermediators between patients and physicians. Therefore, they could take over important parts in evidence-based patient information. At present, diabetes educators are not trained to present and communicate evidence-based information. In this pilot study we tested various course formats on evidence-based diabetology for this target group within a given time frame.
**Aims:** Evaluation of 1 to 3-day course formats in EBHC for diabetes educators.
**Methods:** Since 2003 we collaborate with three institutions which run degree courses for diabetes educators in cooperation with the German Diabetes Society. Special curricula in defined areas of competence have been developed: a) systematic searching in internet-based databases for predefined questions b) critical appraisal of observational and experimental studies c) critical appraisal of presented data / communication with patients/consumers. Four courses with 97 participants have been evaluated with a test instrument in a pre-post-intervention manner. Two independent raters evaluated course results. The test comprises three main elements: 1. Ability to formulate relevant aspects of an intervention study (5 possible points). 2. Ability to calculate effect-sizes (3 points), and 3. Ability to turn numbers into meaningful objective patient oriented communication (1.5 points for criteria of patient information; 2 points for correct calculation; 2 for correct explanation).
**Results:** Trainees found courses very important and useful for their further work. 94 participants completed both tests. The mean score before the course (pretest) was 4.4 (range %11.5) and after the course (posttest) 6.9 (range 3-11.5) (p <0.01). Degree of knowledge gain depended on course duration, in favour of the three days format. Before and after the course participants were able to calculate data correctly. Most was learned in the third aspect of the test, concerning patient information (median pretest/posttest score: 0/2). Although, further analysis showed that participants while being able to understand basic requirements of patient com-

munication, still lack competences in patient-orientated explanation of data.
**Conclusion:** The evaluation instrument is able to measure EBHC competences. For diabetes nurses, who are involved in direct patient consultations and shared decision making, more focus is to be put on communication and explanation of data. A three days course on EBHC seems the minimum to reach the learning objectives. More intensified course formats are necessary to meet the complex needs of diabetes educators.
*Supported by: BMBF*

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**PACCTS (Pro-Active Call Centre Treatment Support) - glycaemic control; a randomised controlled trial in Type 2 diabetes**
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**Background and aims:** Pro-Active call centre based diabetes care has been implemented in several health care systems but its effectiveness has not been evaluated. This study aims to determine whether a Pro-Active call centre, utilising trained non-medical telephonists supported by specially designed software and a diabetes nurse, can improve glycaemic control in Type 2 DM acceptably and cost-effectively.
**Materials and methods:** The study comprises a one year duration, randomised controlled implementation trial including 591 patients [age 67yr (median, range 22-91), duration DM 6yr (1-39)], randomly selected from a population based diabetes information system and randomised to usual care (UC, n=197) or usual care supplemented by PACCTS (n=394). Lifestyle advice and drug treatment in both groups followed local guidelines. PACCTS patients were telephoned according to protocol with the frequency of calls inversely proportional to the last HbA1 c. A postal questionnaire about acceptability was sent to PACCTS patients at 3 mo and 12 mo. Primary outcome: Absolute reduction in HbA1 c. Secondary outcomes: % patients reducing HbA1 c ≥1%; acceptability; satisfaction; cost effectiveness. Analysis was carried out on an 'intention to treat' basis.
**Results:** 332 (84%) PACCTS and 176 (89%) UC patients completed the study; final HbA1 c values were available in 374(95%) PACCTS and 180(92%) UC patients. In PACTTS HbA1 c improved by 0.31% (95% CI (0.11, 0.52); p = 0.003) overall but by 0.49% (0.21-0.77, p<0.001) if baseline HbA1 c >7% and 0% if baseline HbA1 c <7%. The difference in the proportions of patients achieving a ≥1% reduction in HbA1 c significantly favoured the PACCTS intervention: 10% (95% CI 4-16; p<.001) overall, 15% (4-32) if baseline HbA1 c>7%. 65% of questionnaires were returned; more than 90% of PACCTS patients strongly agreed or agreed that the intervention was helpful; they identified convenience, increased knowledge, greater self-efficacy and improved wellbeing as particular benefits. Modelling of the trial findings provided a range of estimates of cost-effectiveness with the current best estimate being £34 k (48 Euros)/QALY.
**Conclusion:** This study demonstrates that Pro-Active Call Centre Treatment Support is very acceptable to people with type 2 diabetes and that it facilitates improved glycaemic control. To achieve the threshold for cost effectiveness commonly employed in England and Wales (about £30 k/QUALY), however, requires either slightly greater improvement in glycaemia or additional components of support (e. g. blood pressure and lipid control). Further, longer term and multiple intervention studies are now underway.
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**The AUDIT Study: regional variations in physician attitudes to diabetic dyslipidaemia**
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**Background and aims:** Controlled clinical trials show that patients with diabetes benefit from lipid-lowering therapy. However, in practice treatment rates remain low and only small numbers of patients reach lipid goals recommended in guidelines.
**Materials and methods:** The Analysis and Understanding of Diabetes and Dyslipidaemia: Improving Treatment (AUDIT) study was a confidential, Internet-based survey which investigated and compared current worldwide clinical practice patterns in the evaluation and treatment of dyslipidaemia in type 2 diabetes. It assessed the prevalence of cardiovascular disease (CVD) and lipid abnormalities, lipid goals physicians aim to achieve, factors influencing lipid goals, guidelines followed, and reasons for poor lipid control. Physicians who treat patients with diabetes from 50 countries across 7 regions (Western Europe, Eastern Europe, Scandinavia, North America, South America, Asia/Pacific and Africa/Middle East) participated. Individual country results were weighted so that accurate results projections could be made in total and by geographical regions.
**Results:** Overall, 2043 diabetes specialists participated in the survey. Physicians reported that a mean of 62% of their type 2 diabetes patients have dyslipidaemia (range: 75% in North America to 49% in Asia/Pacific). A lipid profile was obtained in 91% of all patients (range: 99% in North America to 81% in Eastern Europe). Stated LDL-C, triglyceride and total cholesterol goals were lower for patients with CVD than those without CVD (e. g. 45% of physicians had different LDL-C goals for patients with CVD versus those without CVD) and varied among regions (Table). Physicians estimated that only about half of their patients achieve their lipid goals. Estimated goal attainment rates were reported to be highest in North America (range: 56% for triglycerides to 69% for LDL-C) and lowest in Eastern Europe (range: 43% for LDL-C to 47% for triglycerides). The most common perceived barrier to goal attainment was patient compliance in Western Europe, Scandinavia, North America, and Asia/Pacific versus product access constraints in Eastern Europe, South America and Africa/Middle East. Guidelines had the strongest influence on lipid targets; the guidelines followed varied widely, with most countries following their respective national guidelines. Worldwide, blood pressure control (32%), lipid management (28%), glycaemic control (22%) and smoking cessation (19%) were considered to have the greatest impact on reducing CVD risk; these also differed among regions.
**Conclusion:** AUDIT shows a disparity in dyslipidaemia management in patients with type 2 diabetes with CVD versus those without CVD, suggesting that diabetes is not widely considered a CVD risk equivalent. Regional differences are apparent in lipid goals, barriers to goal attainment, guidelines followed, and the factors believed to have the most influence on CVD.

Table. Mean lipid goals by region (mg/dL)

| Lipid parameter | All regions | Western Europe | Eastern Europe | Scandinavia | North America | South America | Asia/ Pacific | Africa/ Middle East |
|---|---|---|---|---|---|---|---|---|
| LDL-C Patients with CVD | 100 | 101 | 102 | 102 | 93 | 100 | 101 | 98 |
| LDL-C Patients without CVD | 108 | 112 | 108 | 105 | 97 | 108 | 112 | 107 |
| Triglycerides Patients with CVD | 153 | 155 | 150 | 166 | 151 | 145 | 155 | 153 |
| Triglycerides Patients without CVD | 161 | 165 | 156 | 179 | 156 | 150 | 166 | 164 |
| Total cholesterol Patients with CVD | 179 | 179 | 176 | 173 | 175 | 187 | 178 | 175 |
| Total cholesterol Patients without CVD | 188 | 190 | 184 | 180 | 180 | 193 | 192 | 185 |

*This study was sponsored by Pfizer Inc.*

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**A randomised controlled trial of a Diabetes REcall And Management system: results of the DREAM trial**
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**Background and aims:** The opportunity arose to extend the computerised diabetes registers in Easington, North Tees and South Tyneside in the north east of England, to a full structured recall and management system. In evaluating the system with these extended features this study aimed to address the design shortcomings of previous studies of shared care in diabetes,

including the study of Primary Care Trust (PCT) defined areas rather than an unrepresentative sample of general practices, and inclusion of economic assessment. The aim of the trial was therefore to evaluate the effectiveness and efficiency of an area wide 'extended' computerised diabetes register incorporating a full structured recall and management system, actively involving patients and including clinical management prompts to primary care clinicians based on locally-adapted evidence based guidelines.
**Materials and methods:** A cluster randomised controlled trial of 58 GP practices in Easington, North Tees and South Tyneside.
**Results:** Over a 15 month period, patients in intervention practices were significantly more likely to have had a foot check (OR 1.87, 95% CI 1.09, 3.21), dietary advice (OR 2.65, 95% CI 1.17, 6.04), and blood pressure (BP) level (OR 2.28, 95% CI 1.13, 4.59) recorded. Patients in the intervention group were more likely to have at least one diabetes appointment recorded and, when the analytical model allowed for the difference between registers, the intervention effect was significant at the 5% level (OR 2.00 (95% CI 1.02, 3.91)). There were no significant differences in whether eye checks, HbA1 c, cholesterol or creatinine were recorded. The mean cholesterol level in patients from intervention practices was significantly lower than in control practices (mean difference -0.22, 95% CI -0.34, -0.09); When the number of patients on lipid lowering medication at baseline are allowed for in the analytical model, the proportion recorded as on lipid lowering medication at the end of the study was greater in the intervention group than in the control group (relative risk 1.99, 95% CI 1.20 to 3.32). There was no difference in mean HbA1 c or BP level. There were no differences in patient-reported outcomes. The average cost per patient of primary and secondary care diabetes-related visits and telephone consultations in a 12 month period was not significantly different in the intervention group to the control group (UK £310.70 vs UK £299.29).
**Conclusion:** The computerised structured recall and management system improved care for people with diabetes. There was a significant effect on the recording of several key diabetes care activities (BP, foot checks, dietary advice and appointments). Mean cholesterol was also lowered in the intervention group, therefore the intervention effect was not only on recording. This observation was further strengthened by the finding that lipid lowering medication was more likely to be recorded for patients in the intervention group. Subject to sensitivity analyses, costs to the NHS and to patients were not significantly increased by the intervention.
*The project was funded by grants from Diabetes UK and NHS Research and Development.*

# OP 33

# Insulin resistance, metabolic syndrome and cardiovascular disease

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**Adiponectin and soluble IL-2 receptor levels predict progression of coronary artery calcification in Type 1 diabetes**
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**Background and aims:** Insulin resistance and inflammation, including adaptive immunity, promote atherosclerosis. However, little is know concerning the role of these factors in accelerated coronary artery disease in type 1 diabetes (T1DM). We hypothesized that insulin resistance, marked by low plasma adiponectin and T-cell activation, marked by elevated levels of soluble interleukin 2 receptor (sIL-2R) predict progression of coronary atherosclerosis defined by coronary artery calcification (CAC).
**Materials and methods:** Progression of CAC was assessed by electron beam tomography (Imatron C-150 Ultrafast CT) over a 2.6-ys period (range 1.6-3.2) in a cohort of 1,207 T1DM patients and non-diabetic subjects aged 2%55. In a nested case-control study, baseline exam levels of adiponectin and sIL-2R were related to CAC progression in 151 T1DM and 94 non-DM subjects. Cases (n=101) were subjects whose square-root transformed CAC volume increased by >2.5 during follow-up. Controls were frequency matched on gender, age and diabetes. Adiponectin was measured by RIA (Linco Research) and sIL-2R by ELISA (R&D Systems, MN).
**Results:** After adjusting for baseline CAC, age, sex and diabetes status, progression of CAC was significantly related to baseline levels of sIL-2r (OR=1.79, 95% CI 1.16-2.76, p= 0.008 for 1 SD difference in log sIL2r) and adiponectin (OR=0.42; 0-26-0.68 p=0.0004). There was no difference in the effect of sIL-2R between type 1 diabetic patients and non-diabetic subjects (p=0.8 for interaction), however, the protective effect of adiponectin was less pronounced in T1D patients (OR=0.55; 0.50-1.00; p=0.049) compared with non-diabetic subjects (OR=0.24; 0.10-0.58, p=0.001). BMI, waist circumference, CRP, fibrinogen, homocysteine, HbA1 c, HDL, LDL, triglycerides (log), apolipoprotein B, PAI-1 and smoking were not predictive of CAC progression in multiple logistic regression analysis. Adjustment for systolic blood pressure, in addition to baseline CAC, age, sex and diabetes status, weakened but did not remove association between sIL2r and CAC progression (OR=1.54, 95% CI 0.97-2.44, p=0.067), and did not modify the protective effect of adiponectin (OR=0.34; 0.20-0.58 <0.0001). There was no apparent interaction between the effects of adiponectin and sIL-2R on CAC progression.
**Conclusions:** Low adiponectin levels, a novel component of the metabolic syndrome, and soluble IL-2R, marker of adaptive immunity activation predict CAC progression independent of other CVD risk factors in subjects with and without T1DM.

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**Association of hsCRP with later stage β-cell dysfunction and insulin resistance in patients with Type 2 diabetes**
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**Background and aims:** High sensitive C-reactive protein (hsCRP) is discussed to be a potential laboratory marker for endothelial inflammation and cardiovascular risk in patients with and without diabetes mellitus. The current analysis of the cross-sectional IRIS-II study population was performed to investigate the relation between hsCRP and insulin resistance (IR), later stage β-cell dysfunction (βCD), and prevalence of marcovascular complications (MC: apoplex, CAD, MCI) in patients with early stage type 2 diabetes.
**Materials and methods:** The data from non-insulin treated 4270 patients were included in the analysis (2146 male, 2124 female, age (mean±SD): 63.9 ± 11.1 years, BMI: 30.1 ± 5.5 kg/m$^2$, disease duration: 5.4 ± 5.6 years, HbA1 c: 6.8 ± 1.3%). IR was assessed by HOMA score (>2 = resistant) and later stage βCD by measurement of fasting intact proinsulin (reference range < 10 pmol/l).

**Results:** Insulin sensitivity without later stage βCD (group A) was seen in 1042 (24.4%) patients (hsCRP: 4.0 ± 7.4 mg/l, MC: 25.1%), while only 105 (2.4%) sensitive patients had also later stage βCD (group B: hsCRP: 3.7 ± 4.2 mg/l, MC: 33.3%). While 1658 (38.8%) patients had IR without later stage βCD (group C: hsCRP: 5.5 ± 12.3 mg/l, MC: 23.5%), 1465 (34.3%) presented with IR and later stage βCD (group D: hsCRP: 6.8 ± 14.0 mg/l, MC: 31.1%). The mean hsCRP values indicated an overall high cardiovascular risk (> 3 mg/l) in all groups. The stratification of the patients according to the hsCRP-based cardiovascular risk profiles is given in the table. Increasing insulin resistance and increasing β-cell dysfunction were associated with a worsening of the intra-group hsCRP risk distribution. There also was a close correlation between an increase of IR, βCD, and hsCRP with an increased prevalence of macrovascular disease (e.g. A vs. D: p <0.001).
**Conclusion:** The result of this analysis confirm the value of hsCRP to serve as a marker for cardiovascular risk and endothelial inflammation in patients with non-insulin treated type 2 diabetes.

% patients in the different hsCRP risk groups

| Risk level according hsCRP | Group A | Group B | Group C | Group D |
|---|---|---|---|---|
| Low (0–1 mg/l) | 30% | 31% | 19% | 13% |
| Intermediate (1–3 mg/l) | 34% | 59% | 53% | 45% |
| High (3–10 mg/dl) | 27% | 31% | 34% | 38% |
| > 10 mg/dl | 9% | 9% | 15% | 17% |

*This work was supported by a grant of Takeda, Germany.*

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**Prevalence of ischaemic heart disease is strongly associated with insulin resistance and features of metabolic syndrome in subjects yet to develop Type 2 diabetes from the Herts 1931-39 Cohort Study**
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**Background and aims:** Insulin resistance is an important risk factor for the development of cardiovascular disease in patients with Type 2 diabetes. It is also well known that patients with impaired glucose tolerance (IGT) have a greatly increased cardiovascular risk. However, the contribution of insulin resistance to ischaemic heart disease risk needs to be established in subjects who are yet to develop Type 2 diabetes. In this study we examine the relationships between markers of insulin resistance and the metabolic syndrome and the prevalence of IHD in 1179 members of the Hertfordshire Cohort Study without Type 2 diabetes.
**Materials and methods:** Data from 1179 subjects who participated in the Hertfordshire 1931-1939 cohort study clinics (1999–2002), were included for analysis. IHD was defined as the presence of typical angina on the Rose chest pain questionnaire, or the presence of major Q waves on an ECG or previous CABG. Systolic and diastolic blood pressure (DBP) were recorded. HOMA-R, HOMA-S, total cholesterol, LDL-cholesterol, HDL cholesterol, triglycerides were measured. Subjects were screened with an oral glucose tolerance test to establish absence of diabetes. The relationships between these variables and IHD were analysed using logistic regression models with age and gender included as adjustment factors. Odds ratios (95%CI, p-value) are presented for subjects in the highest compared with the lowest third of each variable.
**Results:** The prevalence of IHD was 11% for men and 9% for women. Increased insulin resistance, as determined by HOMA-R, was associated with an increased prevalence of IHD (1.99 (1.24, 3.21) p=0.001). HOMA-B was also associated with an increased IHD risk (1.95 (1.22, 3.12) p=0.002). An increased risk of IHD was also associated with increased triglycerides (2.26 (1.35, 3.77) p<0.001) and high HDL-c was shown to be protective (0.50 (0.30, 0.83) p=0.002). There was no relationship between LDL-c and IHD. Although there were trends for a relationship between IHD and blood pressure, these were not significant. A mutually adjusted model suggested that IHD prevalence was most strongly associated with insulin resistance, (1.65 (1.00, 2.73) p=0.04). Results were similar after adjustment for body mass index.
**Conclusion:** Lipid abnormalities and hypertension are recognised as important risk factors in ischaemic heart disease, and those patients with impaired glucose tolerance yet to develop Type 2 diabetes are well known to be at increased cardiovascular risk. This study demonstrates that insulin resistance and features of the metabolic syndrome are independent risk factors in their own right for IHD even before the development of Type 2 diabetes. It is at least as important to address insulin resistance and features of the metabolic syndrome as it is to target conventional risk factors for IHD.

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**Severity of arterial stenotic changes in Type 2 diabetes patients with coronary artery disease: a significant correlation with decreased insulin sensitivity and higher plasma insulin and plasminogen activator inhibitor 1 levels but not with lipid impairments**
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**Background and aims:** Previous studies have suggested that in nondiabetic patients with coronary artery disease (CAD) the number of stenotic coronary vessels, reflecting the severity of CAD, was significantly influenced by metabolic and fibrinolytic factors. However, these influences remain to be clarified in type 2 diabetic patients with CAD. Therefore, this study was aimed to compare insulin sensitivity, plasma insulin (PI), plasminogen activator inhibitor 1 (PAI-1) and lipoprotein subfraction (total cholesterol (Ch), HDL-Ch, LDL-Ch and triglyceride (Tg)) levels in the following groups of type 2 diabetic patients defined according to angiographically determined number of stenotic coronary arteries (SCAs): (a) 3 SCAs (group A; N=28), (b) 2 SCAs (group B; N=30), (c) 1 SCA (group C, N=24) and (d) without SCAs (group D, N=18) (groups were matched for duration of diabetes and CAD).
**Materials and methods:** In each patient included in the study, CAD was angiographically verified and the SCA was defined as a stenosis with narrowing of the lumen >50% with respect to the pre-stenotic segment while an absence of stenosis was determined when the narrowing of the lumen was < 10%. Insulin sensitivity levels (Si) was determined by minimal model analysis, PI levels were detected by RIA, PAI-1 levels were measured by plasminogen chromogenic plasmin substrate assay and total, HDL-Ch, LDL-Ch and Tg levels by chromatography.
**Results:** We found that Si values were lower in groups A, B and C (A: 0.77+/-0.16; B:1.15+/-0.14 and C:1.41+/-0.17 $min^{-1}/mU/lx10^4$) vs group D (2.11+/-1.4 $min^{-1}/mU/lx10^4$; p<0.05, A vs B vs C:p<0.05). Simultaneously, PI and PAI-1 levels were significantly higher in groups A, B and C compared to group D (PI: A: 31.4+/-3.9; B: 24.5+/-3.7; C: 19.8+/-2.4; D: 12.9+/-2.8 mU/l; A,B,C vs D: p<0.05; A vs B vs C: p<0.05), (PAI-1: A: 8.6+/-1.7; B: 7.1+/-1.0; C: 5.9+/-1.4 D: 3.9+/-1.1 U/ml; A,B,C vs D: p<0.05; A vs B vs C: p<0.05). However, we could not find significant differences in total, HDL-Ch, LDL-Ch and Tg levels between the groups A, B and C. In addition, when all the type 2 diabetic patients with CAD were analyzed together, we found that number of SCAs correlated significantly only with Si values (r=-0.394, p<0.05), PI (r=0.352, p<0.05), and PAI-1 levels (r=0.367, p<0.05).
**Conclusion:** Our results signify that severity of CAD in type 2 diabetics, expressed by the number of SCAs detected angiographically, is associated with increases in insulin resistance, PI and PAI-1 levels. The results imply that insulin resistance influences the severity of CAD mainly through increases in insulinemia and decreases in fibrinolytic activity.

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**After acute myocardial infarction, increased in-hospital mortality and morbidity in patients with metabolic syndrome: major implication of increased blood glucose**
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**Background and aims:** Metabolic Syndrome has been shown to be associated with an increased risk for coronary disease. However, in-hospital mortality after acute Myocardial Infarction (MI) has never been evaluated in patients with Metabolic Syndrome. The aim of the present study was to find out whether Metabolic Syndrome is associated with in-hospital mortality and morbidity.
**Materials and methods:** 457 patients hospitalized with acute MI (assessed on increased plasma troponin levels) from the RICO survey were included in the study. In all patients, presence of Metabolic Syndrome was defined

according to the NECP/ATP III by 3 or more of the following criteria: glycaemia ≥ 6.1 mmol/l, triglycerides ≥ 1.7 mmol/l, HDL-cholesterol < 1.04 (M)/1.29 (F) mmol/l, blood pressure ≥ 130/85 mmHg or waist circumference > 102(M)/88(F) cm.
**Results:** Among the 457 patients, 254 (55%) had Metabolic Syndrome. Renal function, MI characteristics, Left Ventricular Ejection fraction (LVEF) were not different between patients with and without Metabolic Syndrome. In contrast, in the Metabolic Syndrome group, patients were older (67 ± 14 vs. 63 ± 14 years, p<0.01) and percentage of women was higher (34% vs. 18%, p<0.0001). As a major finding, a significant increase of in-hospital mortality (8% vs. 1%, p<0.001) was observed in the patients with Metabolic Syndrome, related to an increase of cardiogenic shock (15% vs. 5%, p=0.002). In contrast, no difference for ventricular arrhythmia and recurrent MI was noted between the 2 groups. In-hospital mortality was associated, in multivariate analysis, with age (p=0.015), LVEF (negatively, p=0.006), Metabolic Syndrome (p=0.013) and anterior MI (p=0.038). Cardiogenic shock was associated, in multivariate analysis, with LVEF (negatively, p<0.0001), ST segment elevation (p=0.018) and Metabolic Syndrome (p=0.019) but not with age, sex, history of coronary disease or anterior necrosis. Among the 254 patients with Metabolic Syndrome, cardiogenic shock was associated, in multivariate analysis, with LVEF (negatively, p=0.002), glycaemia ≥ 6.1 mmol/l (p=0.034), anterior necrosis (p=0.037) and female sex (0.039) but not with lipids, abdominal circumference or blood pressure.
**Conclusion:** 1) Our data indicate for the first time that Metabolic Syndrome is, in patients with MI, independently associated with an increased rate of in-hospital mortality, mainly due to a higher risk for developing cardiogenic shock during in-hospital stay. 2) In patients with Metabolic Syndrome, augmented glycaemia is the metabolic factor associated with the increased risk for cardiogenic shock. Furhter studies are needed to test whether intensive glycaemic control in patients with Metabolic Syndrome may reduce in-hospital mortality and morbidity after MI.

## 198

**Correlation of plasma complement C3 levels with elements of the metabolic syndrome and C3 genotype in patients with coronary artery disease**
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**Background and aims:** Atherothrombotic risk factors (insulin resistance, dysglycaemia, dyslipidaemia, hypertension, hypercoagulability) cluster in subjects with cardiovascular disease. Several lines of evidence suggest that inflammatory molecules, including the complement system, play a role in atherosclerosis and the metabolic syndrome. Previous work has shown that C3 serum levels are elevated in patients with a history of myocardial infarction (MI) and a polymorphic variant of C3 may predispose to MI. The aim of this study was to determine the relationship between plasma C3 levels and features of the insulin resistance syndrome, coronary artery stenosis, MI and C3 genotype.
**Materials and methods:** We measured plasma C3 levels by ELISA in 427 patients undergoing coronary angiography for typical symptoms of coronary artery disease (CAD). Levels were correlated to elements of the metabolic syndrome, coronary artery score and C3 common allelic variants C3F and C3S, determined by PCR-RFLP analysis of genomic DNA.
**Results:** There was a positive correlation between C3 plasma levels and BMI (r=0.353, p<0.001), total cholesterol (r=0.146, p<0.01), triglycerides (r=0.334, p<0.001), glucose (r=0.218, p<0.001), HbA1C (r=0.282, p<0.001) and fibrinogen (r=0.373, p<0.001). In contrast, a negative correlation with HDL was found (r=-0.149, p<0.01). There was no correlation between C3 levels and age, sex, history of smoking, hypertension or coronary artery score. Patients with diabetes (n=25) had higher levels of C3 compared with non-diabetics (1.22 g/l and 1.13 g/l respectively; p=0.02). C3 levels were also higher in patients with a history of MI (n=131) compared with the rest of the group (1.17 g/l and 1.13 g/l respectively; p=0.04). Patients with a history of MI and not on statin treatment (n=34) had the highest C3 levels, whereas patients not on statin treatment without a history of MI (n=170) had the lowest C3 levels (1.23 g/l and 1.10 g/l respectively; p<0.001).
C3 genotype was analysed in 360 individuals and C3S allele frequency was found to be 0.78. C3 levels in individuals with C3SS genotype (n=230) were 1.15 g/l (1.12-1.17), C3SF (n=115) 1.11 g/l (1.08-1.15) and C3FF (n=15) 1.09 g/l (1.01-1.18), a difference that did not reach statistical significance (p=0.12). However, when the C3SF and C3FF individuals were combined, C3 levels were significantly lower 1.11 g/l (1.08-1.14) than C3SS individuals (p=0.046).
**Conclusion:** This work has shown that patients with CAD have high plasma complement levels, which correlate with markers of the metabolic syndrome and a previous history of MI. Also, we have found that C3SS genotype is associated with higher plasma C3 levels, suggesting that C3SS may contribute to the risk of developing CAD.

# OP 34
# Adiponectin

## 199

**Regulation of adiponectin receptor 1 and 2 in human adipose tissue**
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**Background and aims:** Adiponectin is exclusively expressed in adipose tissue and low levels of adiponectin have implications for obesity health problems such as insulin resistance and enhanced risk of artherosclerosis. How adiponectin mediates these effects has been rather obscure, but recently two adiponectin receptors have been identified belonging to the seven transmembrane receptors – AdipoR1 and AdipoR2. AdipoR1 is mainly expressed in muscle cells, whereas AdipoR2 is predominantly expressed in the liver. In the precent study we investigated the presences of these receptors in whole human adipose tissue, in isolated adipocytes and in human adipose tissue during differentiation. In adition we studied these receptors expression regulated by glucose in whole adipose tissue.
**Methods:** Human adipose tissue was obtained from plastic surgery procedures. AdipoR1 and AdipoR2 mRNA were determined by RT-PCR. Adipose tissue fragments were incubated with different concentrations of glucose for 2-72 hours. The influence of the differentiation process was investigated with preadipocytes in primary cultures.
**Results:** AdipoR1 was abundantly expressed in adipose tissue, whereas AdipoR2 mRNA was only detected in minor amounts. AdipoR1 was expressed to the same degree during the adipocyte differentiation process. Glucose was found dose-dependently to reduce the expression of AdipoR1 mRNA - with a reduction of 63% (P<0.01) at a glucose concentration of 35 mM compared with 0 mM.
**Conclusion:** We have identified the expression of AdipoR1 in human adipocytes. We have shown that glucose downregulates this receptor in a dose-dependently way. These findings indicate that adiponectin may have auto/paracrine effects in the adipose tissue. Moreover, high levels of glucose as seen in diabetes may reduce the biological effect of adiponectin by inhibiting the AdipoR1.

## 200

**Autocrine action of adiponectin on human fat cells prevents the release of insulin resistance-inducing factors**
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**Background and aims:** Adipose tissue secretes a variety of factors that influence insulin sensitivity in skeletal muscle. The adipocyte-derived hormone adiponectin is the only known positive regulator of insulin sensitivity and attains high concentrations in blood plasma. In contrast to TNF-alpha plasma adiponectin levels are reduced in diabetic patients. Experiments with insulin resistant animal models revealed that infusion of adiponectin normalises insulin sensitivity.
In the present study, we analysed the capacity of adiponectin to normalise insulin signalling in human skeletal muscle cells co-cultured with human adipocytes or treated with adipocyte-conditioned medium.
**Materials and methods:** Primary human skeletal muscle cells were co-cultured with human fat cells for 48 h or incubated with adipocyte-conditioned medium for 18 h, in the presence or absence of 10 nM adiponectin. The composition of adipocyte supernatants was analysed using cytokine protein arrays.
**Results:** Co-culture with adipocytes or treatment with adipocyte-conditioned medium reduced the insulin stimulated Akt phosphorylation in skeletal muscle cells (50–70%). However, addition of adiponectin to the co-culture prevents the loss of insulin sensitivity. Further, the capacity of adipocyte supernatant to impair insulin signalling in skeletal muscle was abolished when generated in presence of adiponectin. Addition of adiponectin to muscle cells in the absence of adipocyte-conditioned medium had no effect on Akt serine phosphorylation (119 ± 22% for treated vs. control). Concomitant addition of adiponectin and adipocyte-conditioned medium failed to restore normal insulin action in skeletal muscle cells (44 ± 6% of control for supernatant without adiponectin vs. 49 ± 9% for supernatant with adiponectin). To further explore the autocrine regulatory function of adiponectin, we analysed adipocytokines in adipocyte supernatants generated with or without adiponectin. We found that more than 10 cytokines were reduced by at least 50% in response to adiponectin, including IL-6 and IL-8.

**Conclusions:** Our data show that adiponectin can counteract a defect in insulin signalling in human skeletal muscle cells co-cultured with adipocytes. This is unrelated to direct effects of adiponectin on insulin sensitivity in the myocytes. We therefore suggest that adiponectin operates as an autocrine regulator of adipocyte secretion including adipocytokines like IL-6 and IL-8.

## 201

**Hyposecretion of adiponectin in obesity: contribution of stromal-vascular cells of adipose tissue.**

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**Background and aims:** Adiponectin (ApN) enhances insulin sensitivity, controls fuel homeostasis and protects against atherosclerosis. Although hypoadiponectinemia plays a determinant role in metabolic disorders associated with visceral obesity, the mechanisms responsible for low ApN levels in obese subjects are still unclear. In this study, we compared ApN secretion by cultured explants (tissue fragments) or isolated adipocytes obtained from visceral fat of lean (L) or obese (O) subjects. We focused on the potential modulatory role of the stromal-vascular cells (SVC) on ApN secretion by adipose cells.
**Materials and methods:** Visceral adipose tissue (and plasma) were sampled from 19 O (10 women and 9 men; age: 45 ± 3 y; BMI: 40.9 ± 1.6 kg/m$^2$) and 18 L subjects (7 women and 11 men; age: 59 ± 3 y; BMI: 24.1 ± 0.6 kg/m$^2$) undergoing abdominal elective surgery. Explants and isolated adipocytes were cultured for up to 24 h in basal MEM medium. Adipocytes were cultured either alone or with their SVC in this medium, or in different conditioned media (previously obtained by culturing independently SVC from L or O subjects for 24 h). We measured ApN and leptin secretion in medium by RIA and ApN mRNA by real time RT-PCR.
**Results:** ApN secretion by visceral adipose explants was not influenced by gender or age, but was positively correlated with plasma ApN and negatively with BMI ($P < 0.05$). ApN secretion by O explants (ng/mg adipose tissue) was lower than that by L ones from 8 h of culture onwards ($P = 0.012$) and a 2-fold decrease was observed at 24 h ($P < 0.001$). Surprisingly, there was no significant difference in ApN secretion by isolated adipocytes of O and L subjects (ng/ml packed fat cells). Such discrepancy persisted when data were expressed perμg fat cell DNA and was not explained by difference in ApN mRNA abundance. In the same conditions, leptin secretion by explants or adipocytes was elevated in obesity ($P < 0.05$). Because of the difference between ApN secretion by O explants and adipocytes, we examined the potential contribution of SVC to decreased ApN secretion in obesity. First, we excluded the possibility that visceral SVC released detectable amount of ApN during 24 h-cultures. Next, we measured ApN secretion by adipocytes cultured either alone or with their SVC. Co-culturing adipocytes and SVC from L subjects increased ApN release in medium by 35% ($P < 0.001$). However, the presence of SVC did not influence ApN release by O adipocytes. This could be explained either by the fact that O SVC did not exert any stimulatory effect or that O adipocytes were resistant to this stimulation. To test these hypotheses, we cultured L or O adipocytes in medium conditioned by SVC. When adipocytes from L subjects were cultured in medium conditioned by O (or L) SVC, ApN secretion was increased by 30% as compared to basal conditions ($P < 0.05$). By contrast, culturing O adipocytes in medium conditioned by O or L SVC did not modify basal ApN secretion.
**Conclusion:** ApN secretion by cultured explants but not adipocytes is decreased in O subjects. These results, alongside experiments of adipocytes cultured with their SVC or in different conditioned media, suggest that a factor released by SVC is able to stimulate ApN secretion by L adipocytes while O adipocytes are resistant to this factor. This resistance might contribute to hypoadiponectinemia observed in obesity.

## 202

**Chronic underexpression of adiponectin in transgenic mice**

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**Background and aims:** Adiponectin (ApN) is an adipocyte-derived hormone with multiple biological functions. Its expression is reduced in obesity, type 2 diabetes and cardiovascular disease. Administration of this hormone produces weight loss, improves insulin sensitivity and glucose tolerance in animal models of obesity and diabetes. In this study, we investigated the effects of chronic underexpression of full length ApN in a transgenic (Tg) model of mice without overt obesity, type 2 diabetes or cardiovascular disease mice.
**Materials and methods:** We created two heterozygous Tg mouse lines that underexpress the mouse ApN gene specifically in adipose tissue (on a pure FVB or a mixed FVB/C57Bl background). Tg and wildtype (WT) littermates were studied. Fat pads from different depots were collected at 3 months of age for determination of adipose tissue weight, mRNA levels and ApN content in females only (males being kept for mating and transgene propagation). Blood was collected at 2 weeks and 3 months of age in both male and female mice for determination of plasma ApN levels. Body weight was monitored throughout the study. ApN levels were measured by RIA and mRNA abundance by real time RT-PCR.
**Results:** When compared to WT littermates, ApN mRNA levels were reduced by 20–30% in the perigonadal (ovarian) fat depot of 3 month-old Tg females from both mouse lines ($p < 0.001$). Accordingly, the ApN content in perigonadal adipose tissue homogenates was decreased by 36% ($p < 0.05$). ApN mRNA levels were not modified in other depots (retrovesical-visceral and inguinal).
Plasma ApN levels of 3 month-old Tg females were not different from those of WT mice, while a significant reduction was observed in 3-month-old Tg males (23%, $p < 0.05$). However, earlier in the course of the study, at 2 weeks of age, plasma ApN levels were markedly decreased in both Tg females and males (34%, $p < 0.05$ and 40%, $p < 0.01$ *vs.* WT, respectively).
Body weight of Tg males or females, at 2 weeks or 3 months of age, did not differ from that of WT mice. However, weight of perigonadal adipose tissue was increased by 2-fold ($p < 0.05$) in 3 month-old Tg females when compared to WT littermates, a rise that did not occur in the other fat depots.
Decreased ApN levels and increased weight of perigonadal fat tissue in Tg mice were associated with diminished expression of uncoupling protein 2 (UCP2) involved in energy dissipation (–37%, $p < 0.05$), and increased expression of a key enzyme involved in lipogenesis (2.5 -fold for fatty acid synthase (FAS), $p < 0.05$). A 1.75 -fold increase in TNF alpha expression ($p < 0.01$) was also observed in perigonadal tissue of these mice. The underexpression of ApN did not affect the expression of other messengers (Glut4, aP2, PPARγ2, PPARα, ACO, SREBP1)
**Conclusion:** Chronic underexpression of ApN in one adipose site, even when associated with transient systemic repercussion, resulted in local increase of adipose tissue weight, accompanied by downregulation of UCP2 mRNAs and upregulation of FAS and TNFalpha mRNAs. Other key adipose molecules tested were unchanged.

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**Insulin and leptin effects on resistin, TNFα and IL1β mRNA and protein production from peripheral monocytes**

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**Background and aims:** Inflammatory cytokines, circulating and locally produced in the arterial wall, are thought to be involved in the atherogenic process. A major source of circulating cytokines are the peripheral mononuclear-monocytic cells. Resistin is also expressed in human peripheral monocytes at levels much higher than in adipocytes, is induced by iflammatory stimuli and can have direct effects on endothelial cells by activating endothelin and cell adhesion molecules. We examined whether the monocyte mRNA and protein levels of resistin, TNFα and IL1β are altered when exposed to high levels of insulin and leptin, commonly seen in obesity.
**Materials and methods:** We studied 9 healthy subjects (5 females and 4 males, BMI: 25.3 ± 0.7, Age: 34.7 ± 0.8). Peripheral monocytes were isolated from total blood by Ficoll-Paque and selective adherence on Petri dishes and were exposed to insulin (2IU/L) or leptin (100ng/ml) or both together. We measured the relative mRNA levels of resistin, TNFα and IL1β by real-time quantitative RT-PCR (Light Cycler, Roche) and calculated them as the relative ratio of the fluorescence acquisition of the corresponding mRNA divided by that of β-actin, using the Relative Quantification Software (Roche). We also measured fasting insulin (RIA), glucose and FFA levels. Monocyte secreted protein levels were measured by Elisa (BioVendor Lab for resistin or R&D Systems, for TNFα and IL1β).
**Results:** Leptin increases resistin, TNFα and IL1β mRNA and protein production from peripheral monocytes (Table). In contrast, insulin does not appear to have any stimulating effect or to compromise the stimulatory effects of leptin when combined together (Table).

| | Resistin mRNA | TNFα mRNA | IL1β mRNA | Resistin ng/ml | TNFα pg/ml | IL1β pg/ml |
|---|---|---|---|---|---|---|
| Control | 4.7 ± 0.9 | 0.17 ± 0.03 | 43.0 ± 10.4 | 0.27 ± 0.06 | 142.0 ± 11.5 | 86.7 ± 14.9 |
| Insulin | 2.3 ± 0.5 | 0.27 ± 0.06 | 42.3 ± 8.7 | 0.30 ± 0.05 | 159.5 ± 12.5 | 94.8 ± 14.1 |
| Leptin | 8.6 ± 1.8 | 0.36 ± 0.06* | 67.3 ± 23.7 | 0.39 ± 0.06 | 167.5 ± 14.6 | 113.4 ± 21.4 |
| Ins+lep | 6.3 ± 2.0 | 0.27 ± 0.05 | 52.9 ± 9.9 | 0.44 ± 0.06* | 167.1 ± 13.5 | 114.8 ± 22.7 |

*, $p<0.05$ vs control

**Conclusion:** Leptin, but not insulin, at high concentrations stimulates mRNA and protein production of the inflammatory cytokines TNF-α and IL-1β and of the adipokine resistin from human peripheral mononuclear cells *in vitro*. This might provide an important coupling mechanism between obesity and atherosclerosis.

## 204

**Effects of inhibition of insulin-stimulated glucose metabolism on leptin production and secretion**

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**Background and aims:** Acute leptin secretion in response to nutritional stimuli is related to insulin-stimulated glucose metabolism and insulin may also play a general anabolic role on leptin protein production. Basal leptin levels are increased with increasing adiposity, but the acute leptin secretion response to nutritional stimuli may be suppressed if insulin-stimulated glucose metabolism is reduced, as it is in obese and insulin resistant animals. The aim was to determine if insulin stimulation of leptin production and secretion from isolated epididymal adipocytes was maintained when insulin-stimulated glucose flux (ISGF) was reduced.
**Materials and methods:** Basal and insulin-stimulated leptin metabolism was studied in epididymal adipocytes from lean rats to examine the normal acute leptin response to nutritional stimuli over 2 hours. Suppression of ISGF was achieved by (1) the inhibitor cytochalasin B (CB), and (2) fasting the rats prior to adipocyte isolation. Insulin stimulation and suppression of ISGF was confirmed by measurement of glucose metabolism. Leptin secretion and adipocyte *ob* mRNA and leptin protein content were measured. Leptin secretion plus content was used as a measure of total leptin production during the 2 hours.
**Results:** Leptin secretion was stimulated above basal following incubation with insulin (3.7-fold, $p<0.001$) but was reversed when ISGF was inhibited by co-incubation with insulin and CB ($p<0.0001$ compared to insulin, *ns* compared to basal). Similarly, no insulin-stimulated increase in leptin secretion was seen from fasted adipocytes which had suppressed ISGF. Incubation with insulin caused a 1.8-fold increase in leptin production compared to basal ($p<0.05$) which was partially maintained in the presence of CB (1.6-fold over basal). Total leptin production was lower in adipocytes from fasted rats (as previously reported, $p<0.001$) but incubation with insulin produced a 2-fold increase in the amount of leptin produced compared to basal ($p<0.05$). *Ob* mRNA was decreased in the fasted adipocytes ($p<0.01$) but was not stimulated concordantly with leptin production by incubation with insulin, and was unaffected by incubation with CB.
**Conclusion:** Insulin increases leptin production, whereas inhibition of ISGF through fasting or with the inhibitor CB reverses the insulin-stimulated increase in leptin secretion. The acute increases in total leptin production were not accounted for by an increase in *ob* expression. These models provide evidence that leptin production can be maintained in the presence of high insulin, but increased ISGF is necessary to stimulate acute leptin secretion in response to nutritional stimuli.

# OP 35
# Exocytosis and ion channels

## 205

**Sequential insulin exocytosis and redistribution of SNAP25 analyzed with two-photon imaging**

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**Background and aims:** Secretory cells often exhibit exocytic events that involve more than one granule, which have been referred to as "compound exocytosis." These events, however, include two distinct phenomena, sequential exocytosis and multigranular exocytosis. In sequential exocytosis, vesicles fuse selectively with other vesicles that have already fused with the plasma membrane. In contrast, in multigranular exocytosis, multiple vesicles fuse with each other before exocytosis. Quantitation of these two types of compound exocytosis has been difficult with classical methodologies due to the lack of spatial information. We here investigated insulin granule exocytosis in intact pancreatic islets and dynamics of t-SNARE, SNAP25, during individual exocytic events.
**Materials and methods:** Pancreatic islets were isolated from mice by collagenase digestion and were immersed in a solution containing polar fluorescent tracers, sulforhodamine B. The tracer was excited by laser-scanning microscopy with a mode-locked femtosecond-pulse Ti:sapphire laser (wavelength, 830nm), and islets were stimulated with 20 mM glucose. Exocytic event of individual insulin granule could be identified as the appearance of small fluorescent spot (mean diameter: 420 nm), reflecting the entry of extracellular polar fluorescent tracer into the fused vesicle, as previously reported. The occurrence of sequential exocytosis or multigranular exocytosis was quantified, respectively. In the experiments where dynamics of SNAP25 was investigated, we constructed an adenoviral vector encoding enhanced cyan fluorescent protein (eCFP)-SNAP25 fusion protein. Islets were observed with two-photon microscopy 12–24 hours after the transfection, and fluorescence of eCFP or SRB was simultaneously detected at 400–490 nm or 550–650 nm, respectively.
**Results:** More than 50% of exocytosis was sequential in exocrine pancreatic acinar cells studies with the same approach. In contrast, sequential exocytosis was found to account for <5% of exocytic events in β cells stimulated either with glucose in the absence or presence of forskolin and PMA. The occurrence of sequential exocytosis did not increase when stimulated with photolysis of a caged-$Ca^{2+}$ compound or with 50 mM KCl. Multigranular exocytosis was also rarely found, as the distribution of fluorescent intensity of omega profiles showed single component. We next detected redistribution of eCFP-SNAP25 from the plasma membrane into the membrane of the fused granule, and found that it occurred in a large proportion (54%) of sequential exocytic events, but in only a small fraction (5%) of solitary fusion events. In the case of sequential events, second exocytosis occurred 16.9 ± 21.2 s (n =19) after the onset of eCFP-SNAP25 redistribution into the granule membrane. We occasionally found that removal of cholesterol in the plasma membrane by methyl-β-cyclodextrin facilitated both redistribution of eCFP-SNAP25 and sequential exocytosis by threefold.
**Conclusion:** Our observations support the hypothesis that SNAP25 is a plasma membrane factor responsible for sequential exocytosis. Rare redistribution of SNAP25 may prevent β-cells from sequential exocytosis. The suppression of sequential exocytosis forces β-cells to transport insulin granules to the cell surface for exocytosis, and may make insulin exocytosis more sensitive to metabolic states of β-cells.

## 206

**The synaptotagmin VII spliced variants α, β and Δ are expressed in native islet β-cells and regulate insulin exocytosis**

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**Background and aims:** The synaptotagmins (SYT) are a family of calcium-binding proteins that participate in the regulated exocytosis of vesicles. Most of the 15 members share a common structure composed of a short intravesicular NH2-terminal region, a single membrane spanning domain, a linker region, and two homologous C2 domains (C2A and C2B) important for calcium and phospholipid binding. We have previously demonstrated that SYTI to IX isoforms are expressed in a variety of insulin-producing cell lines. However, only SYTIX is associated with insulin secretory granules in

native β-cells and is implicated in exocytosis. In addition, SYTVII has been proposed to regulate insulin secretion but its subcellular location (plasma membrane versus vesicles) and the number of alternative spliced variants expressed in β-cells (single or multiple forms) are controversial. The aim of the current study was to establish the expression pattern, localization and function of SYTVII splice variants in insulin-producing cells.
**Materials and methods:** RT-PCR analysis using SYTVII exon-specific primers was performed on RNA isolated from the clonal β-cell line INS-1E as well as from rat brain, islets and purified β- and α-cells. We confirmed translation of the various spliced variants by Western blot analysis and assessed protein subcellular localization by confocal microscopy. RNA interference was employed to determine the functional impact of SYTVII on insulin secretion.
**Results:** SYTVII α, β and a novel spliced variant lacking exons 9 to 14 (C2AB domains) were found to be the most abundant transcripts expressed in islet β-cells as well as in INS-1E cells. In contrast, glucagon-producing α-cells only expressed the SYTVII α mRNA. Western blot analysis revealed that INS-1E cells predominantly produced the SYTVII α variant. Endogenous protein co-localized with both synaptic-like microvesicles and insulin granules. Over-expression of SYTVII Δ revealed a vesicular distribution different to that of insulin granules while over-expression of SYTVII α which co-localized with insulin resulted in a marked decrease of insulin granules with a concomitant increase of the isoform at the plasma membrane. Suppression of SYTVII using targeted shRNA inhibited stimulated-insulin secretion.
**Conclusion:** Our results demonstrate that SYTVII is expressed in β-cells and regulates insulin exocytosis. Furthermore, spliced variants have distinct subcellular localization indicating different spatio-temporal functions in this process.
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**Independent roles for glucose and insulin in the control of glucagon secretion revealed by imaging ATP and $Ca^{2+}$ with recombinant targeted probes**
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**Background and aims:** Progressive loss of the normal control of glucagon secretion is frequently observed in type 1 diabetes and contributes to an increasing risk of hypoglycaemic episodes. The mechanisms leading to the inhibition of glucagon secretion from pancreatic α-cells are presently unresolved. However, the investigation of changes in nutrient metabolism and free cytosolic calcium concentration ($[Ca^{2+}]_c$) in α-cells is complicated by the fact that these cells are much less abundant in the islet than β-cells. Our aims here were to: (1) develop recombinant targeted probes, delivered using adenoviral vectors, to image changes in free [ATP] and $[Ca^{2+}]_c$ selectively in primary α-cells and in the glucagon secreting cell line, αTC1-9; (2) to assess the relative importance of glucose, insulin and zinc in the regulation of glucagon secretion.
**Materials and methods:** Recombinant adenoviruses encoding the fluorescent cytosolic $Ca^{2+}$ probe, Pericam or humanised firefly luciferase, were generated by fusing cDNA encoding each probe respectively downstream of a 1.6 Kb fragment of the rat preproglucagon promoter. After 2 - 4 days of infection of intact mouse islets or αTC1-9 cells with the adenoviruses, imaging was performed using either a TILL photonics fluorescence microscope (Pericam; 40x oil immersion objective) or a Photek intensified photon-counting camera (luciferase; 10x air objective). Glucagon secretion was measured by radioimmunoassay.
**Results:** Immunocytochemistry revealed that both the targeted Pericam and luciferase were expressed efficiently and specifically in α-cells within the intact islet and in isolated α-cells. Increases in glucose concentration (0,1 and 20 mM) caused elevations of free cytosolic [ATP] (5–20%) as expected ($p<0.05$) in α-cells. Isolated mouse α-cells displayed oscillations of $[Ca^{2+}]_c$ at 0.5 mM glucose and responded to 30 mM KCl with an increase in $[Ca^{2+}]_c$. These oscillations were either inhibited or decreased in the presence of high glucose concentration. Similarly, 50% of αTC1-9 cells displayed $[Ca^{2+}]_c$ oscillations in the absence of glucose which were suppressed by blockade of voltage-sensitive (L-type) $Ca^{2+}$ channels, whilst 30 mM KCl elicited $[Ca^{2+}]_c$ increases in 95% of cells. An increase in glucose concentration from 0 or 1 mM to 20 mM suppressed completely, or significantly ($p<0.01$) decreased, the frequency of $[Ca^{2+}]_c$ oscillations, without affecting their amplitude. Glucagon secretion was inhibited in parallel with these changes. Added insulin dose-dependently (1.7, 3.4 and 17 nM) decreased the frequency of $[Ca^{2+}]_c$ oscillations in αTC1-9 cells at either 0 or 0.5 mM glucose ($p<0.01$), but not at 20 mM glucose. The effects of insulin were suppressed by 100 nM wortmannin or 50 μM LY294002, implicating a role for phosphatidylinositol 3' kinase signalling. By contrast, addition of insulin-like growth factor-1 (IGF-1; 10 nM) had no effect on the $[Ca^{2+}]_c$ oscillations at any glucose concentration tested. Moreover, zinc ions (0.7 nM–30 μM) did not affect the frequency of $[Ca^{2+}]_c$ oscillations, either in the presence or absence of insulin, arguing against a role for these ions as direct inhibitors of glucagon secretion.
**Conclusion:** Both primary α-cells and clonal αTC1-9 display $[Ca^{2+}]_c$ oscillations at low glucose concentrations. These oscillations are modulated directly by both glucose, presumably acting to increase intracellular ATP levels and inhibit ATP-sensitive $K^+$ channels, and by insulin, but are not affected by IGF-1 or zinc ions.
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## 208

**Involvement of BK channels in regulation of glucose homeostasis**
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**Background and aims:** In pancreatic beta-cells besides $K_{ATP}$ channels voltage-activated $Ca^{2+}$ and $K^+$ channels play an important role for glucose-induced electrical activity. It is still a matter of debate whether BK channels are involved in regulation of membrane potential (Vm) and thus insulin secretion. In this study we used BK channel-deficient ($BK^{-/-}$) mice to elucidate the contribution of BK channels to stimulus-secretion coupling and maintenance of glucose homeostasis.
**Materials and methods:** For glucose and insulin tolerance tests 2 g glucose /kg body weight (BW) and 0.6 I.U. insulin/kg BW, respectively, were injected i.p. in male $BK^{-/-}$ and wildtype (WT) littermates. Vm and ion currents were measured with the patch-clamp technique. Cytosolic free $Ca^{2+}$ concentration ($[Ca^{2+}]_c$) was assessed by fura-2. Insulin secretion was determined by radioimmunoassay. For immunostaining of BK channels a specific antibody targeted against the C-terminus of the α-subunit was used.
**Results:** 12 weeks old $BK^{-/-}$ mice were significantly more glucose tolerant than their littermates (120 min blood glucose concentration (BGC): $BK^{-/-}$: 3.9 ± 0.3 mM, WT: 7.6 ± 0.3 mM; $p<0.001$; n=8) pointing to either increased insulin secretion or sensitivity in $BK^{-/-}$ mice. Since the drop of BGC following i.p. injection of insulin was similar in $BK^{-/-}$ and WT mice (decrease to 37.7 ± 7.6%, n=6, vs. 34.3 ± 3.9%; n=7; respectively) the improved glucose tolerance seems not to be a result of increased insulin efficacy. Therefore we investigated whether ablation of BK channels alters pancreatic beta-cell function. In WT islets BK channels were detectable by a specific antibody. Large conductance $K^+$ channels sensitive to $Ca^{2+}$ (Po at −50 mV: 0.012 ± 0.002, n=6; cord conductance: 217 ± 22 pS, n=5) were present in 16 out of 43 inside-out patches of WT beta-cells but absent in all patches of $BK^{-/-}$ cells (n=30). Depletion of BK channels neither shifted glucose dependency of electrical activity (n=10) or insulin secretion (n=5) nor altered the triphasic response of $[Ca^{2+}]_c$ induced by stimulation with 15 mM glucose (n=14). Analysis of glucose-induced action potentials (APs) revealed that loss of BK channels led to a significant increase in full width at half maximum amplitude (18 ± 1 ms vs. 12 ± 1 ms, n=12, $p<0.001$). In addition, the characteristic afterhyperpolarization was lacking in APs of $BK^{-/-}$ beta-cells. In WT cells identical changes in AP waveform were observed with 100 nM iberiotoxin (n=4). To elucidate the role of BK channels in oxidative stress whole-cell recordings of $K^+$ currents induced by 1 mM $H_2O_2$ were performed. We could show that in WT beta-cells the current activated by the oxidant comprised a tolbutamide-insensitive component of 8.3 ± 1.5 pA (n=4) that was inhibitable by 10 μM paxilline. By contrast, in $BK^{-/-}$ cells the $H_2O_2$-induced current was completely blocked by the sulfonylurea (n=4).
**Conclusions:** BK channels are present in pancreatic beta-cells and contribute to electrical activity evoked by glucose. Although constitutive knock-out of BK channels significantly improves glucose tolerance, glucose dependency of Vm, $[Ca^{2+}]_c$ and insulin secretion are not drastically altered in $BK^{-/-}$ beta-cells. We speculate that the influence of BK channels may become more prominent under conditions of oxidative stress.
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**The $Ca^{2+}$-ATPase SERCA3 influences membrane potential and the pattern of cytosolic $[Ca^{2+}]_c$ oscillations in glucose -stimulated mouse β-cells**

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**Background and aims:** The low-affinity $Ca^{2+}$-ATPase SERCA3 is highly expressed in β-cells. It mediates uptake of cytosolic $Ca^{2+}$ by the endoplasmic reticulum during each period of $Ca^{2+}$ influx from the extracellular medium, whereas the high-affinity SERCA2b controls basal $[Ca^{2+}]_c$. For unknown reasons, β-cells within mouse islets display variable patterns of $[Ca^{2+}]_c$ oscillations during glucose (G) stimulation. These oscillations can be either regular and fast, or mixed with rapid oscillations superimposed on slow ones. We studied the role of the membrane potential (MP) and the possible intervention of SERCA3 in the generation of both types of $[Ca^{2+}]_c$ oscillations.
**Materials and methods:** All experiments were performed with single islets from normal or SERCA3 KO mice. MP was measured with intracellular microelectrodes. In some experiments, $[Ca^{2+}]_c$ (fura PE3) was monitored simultaneously with MP or insulin release. The expression of SERCA isoforms was studied by semiquantitative RT-PCR and real-time PCR.
**Results:** Control islets stimulated with 7-15 mmol/l G displayed rapid and regular (3–4.$min^{-1}$), or mixed (0.2-0.3.$min^{-1}$) $[Ca^{2+}]_c$ oscillations. Mixed $[Ca^{2+}]_c$ oscillations were observed in more than 50% of the islets stimulated with 8 mmol/l G. Simultaneous recordings of MP in single β-cells within an islet and $[Ca^{2+}]_c$ in the whole islet demonstrated that the mixed pattern did not result from the coexistence of two subpopulations of β-cells with distinct patterns of electrical activity, but from a peculiar electrical activity in each β-cell of the islet: a series of rapid MP oscillations separated by prolonged silent intervals. Each slow $[Ca^{2+}]_c$ increase during mixed oscillations was due to a progressive summation of fast oscillations. Simultaneous measurements of $[Ca^{2+}]_c$ and insulin release revealed that each mixed $[Ca^{2+}]_c$ oscillation triggered a synchronous oscillation of insulin secretion. The role of SERCA3 in the control of MP and $[Ca^{2+}]_c$ was evaluated using islets from SERCA3 KO mice. Ablation of SERCA3 did not alter the expression of other SERCAs as compared with control islets: no expression of SERCA1a or 1b, weak expression of SERCA2a and clear expression of SERCA2b. During stimulation with 7–15 mmol/l G the electrical activity of SERCA3 KO β-cells was different from that of control β-cells. Thus, MP oscillations were characterized by a lower frequency (average -60%) but larger depolarization phase (average +70%). Importantly, MP oscillations were consistently regular. The peculiar pattern with changes in the frequency was never observed, nor was the mixed pattern of $[Ca^{2+}]_c$ oscillations.
**Conclusion:** The regular or mixed $[Ca^{2+}]_c$ oscillations in G-stimulated islets result from two distinct patterns of electrical activity in all β-cells of the islet. Disruption of SERCA3 abolishes both the periodic electrical activity and the mixed $[Ca^{2+}]_c$ oscillations, and augments β-cell depolarization. These results suggest that the endoplasmic reticulum participates in the control of the β-cell MP during G stimulation.

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**Ryanodine receptors and transient receptor potential channels provide a distinct mechanism for depolarization of β-cells**

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**Background and aims:** Depolarization of plasma membrane potential from about -70 to about -40 mV is a critical event in the process of stimulation of insulin secretion by glucose and incretin hormones. Closure of the $K_{ATP}$ channels is widely known to mediate such depolarization. However, closure of the $K_{ATP}$ channels alone cannot fully account for the depolarization in this range unless there is a depolarizing inward current. It has been demonstrated that β-cells have transient receptor potential (TRP) superfamily of cation channels as well as intracellular $Ca^{2+}$ channels like the ryanodine (RY) receptors. It is unclear how these channels contribute to the signalling mechanisms involved in stimulus-secretion coupling. We investigated whether activation of TRP channels could mediate membrane depolarization and we have identified a mechanism that might activate the TRP channels.
**Materials and methods:** $[Ca^{2+}]_i$ was measured from fura-2 loaded single rat insulinoma cells (S5 cells) derived from INS-1 cells using a microscope-based fluorescence system from PTI. Membrane potential was measured by perforated-patch whole cell configuration of the patch-clamp technique.
**Results:** We first activated ryanodine receptors by 9-methyl, 5,7-dibromo eudistomin D (MBED) and found that this resulted in a prolonged $[Ca^{2+}]_i$-plateau, in addition to the $Ca^{2+}$ release from the endoplasmic reticulum (ER) and $Ca^{2+}$-induced $Ca^{2+}$ release. The $[Ca^{2+}]_i$-plateau disappeared on ommission of $Ca^{2+}$ from the extracellular medium suggesting that the plateau represents $Ca^{2+}$ entry across the plasma membrane. We studied properties of the $Ca^{2+}$ entry pathways by studying the effects of pharmacological tools on the $[Ca^{2+}]_i$. These results further suggest that activation of the RY receptors can cause activation of a group of $Ca^{2+}$-permeable channels located on the plasma membrane. The consequence of activation of these plasma membrane $Ca^{2+}$ channels on the membrane potential was investigated by patch-clamp technique. Activation of RY receptors and consequent activation of the plasma membrane $Ca^{2+}$ channels depolarized membrane potential from -70 mV to -40 mV. The $Ca^{2+}$entry was blocked by membrane depolarization, SKF 96365, 2-aminoethoxydiphenylborate and $LaCl^{3+}$. Rather surprizingly, it was not altered by $GdCl^{3+}$. $Ca^{2+}$-entry was not blocked by nimodipine and was not altered by diazoxide. $Ca^{2+}$ entry was observed even when the ER $Ca^{2+}$ pool was emptied by prior treatment with thapsigargin. Replacement of extracellular $Na^+$ by choline or prior application of carbachol did nor alter $Ca^{2+}$ entry. $Ca^{2+}$ entry deactivated, apparently spontaneously after about five minutes and was activated again in an oscillatory nanner.
**Conclusion:** The properties of the $Ca^{2+}$ entry demonstrated in this study suggests that it is likely mediated trrough the TRP channels. Such $Ca^{2+}$ entry depolarizes membrane potential even when $K_{ATP}$ channels are opened by diazoxide. Furthermore, we demonstrate that the presumptive TRP channels can be activated as a consequence of activation of RY receptors. This activation is not essentially dependent on the filling state of the ER and could be due mediated by coupling of RY receptors to the TRP channels. Our results suggest that RY receptors and the TRP channels provide a distinct mechanism for membrane depolarization within the critical range of -70 mV to -40 mV. This mechanism may be an important component of signal transduction and stimulus-secretion coupling in β-cells.
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# OP 36

# Genetics of Type 2 diabetes

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**EIF4α2 on chromosome 3q27 is a positional candidate gene for Type 2 diabetes**

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**Background and aims:** Genetic variations at the 3q27 locus are linked to type 2 diabetes (T2D) in French and Japanese families and modulate quantitative traits for insulin resistance and the metabolic syndrome in several populations. The *APM1* adiponectin gene maps to this linkage region, and was first extensively screened for rare exonic mutations and common variants. A haplotype of two promoter variants associate with T2D but no *APM1* SNP or haplotype accounts for the linkage finding. Aiming at deciphering the diabetogenic variants at this locus, we have investigated the coding and regulatory sequences for positional candidates spanning a 460 kb genomic region: *AHSG* (α-2-HS-Glycoprotein), *KNG* (kininogen), *EIF4α2* (Eukaryotic Initiation Factor 4α2), *RFC4* (Replication Factor C4) and *SIAT1* (sialyltransferase 1). For a subset of variants validated in our diabetic samples, we have conducted both familial and case-control analyses in order to test the effect of common variants or haplotypes on T2D risk and contribution to the linkage in French families.
**Materials and methods:** From 60 SNPs identified in a first set of 40 linked family members, the 27 most informative were analysed in the whole family sample (432 overt diabetes/72 IGT/129 normoglycaemic individuals) to detect an association with diabetes and linkage using FBAT, and with age of T2D onset as a quantitative trait (mean age: 49.5 ± 10.6, range:23-83). The association with T2D was also tested in 311 diabetic and 221 normoglycaemic subjects. Gel shift experiments were performed to assess a functional effect of associated variants located in potentially regulatory sequences.
**Results:** Five SNPs are potentially associated ($p<0.10$) to early onset diabetes (<45 yrs) in T2D families and only one SNP in EIF4α2 gene showed significant difference of allele frequencies between diabetic cases and controls (25% *vs* 18%, $p=0.02$). This SNP1 is also associated within the families with age at T2D onset (QTDT, $p=0.002$) and with evidence of linkage (lod-score > 5 in sib-pairs concordant for the minor allele, $p<0.05$). Additional variants of the putative EIF4α2 promoter were tested in the same groups to assess if a SNP haplotype could better explain both association and linkage at this locus. A single promoter variant SNP2 in tight linkage disequilibrium with SNP1 ($\delta=0.973$) was shown to be more strongly associated in the family sample with diabetes status ($p=0.003$), age at onset ($p=6.10^{-4}$) and linkage ($p=0.02$). No haplotype estimated from all frequent SNPs (>5%) tested in this study gave more significance for association or linkage with diabetes. Moreover, no interaction between these associated SNPs and *APM1* variants was displayed in a preliminary analysis. We are also investigating the functional impact of EIF4α2 variants by a molecular approach and are analyzing more variants which may contribute to linkage as well.
**Conclusion:** Our results provide suggestive genetic evidence for a role of EIF4α2 in the regulation of glucose homeostasis. This factor, ubiquitously expressed with controlled levels in pancreas and adipocyte, is part of the EIF4F complex which is regulated by insulin, glucose and amino acids via the Akt/mTOR pathway and PHAS-1 phosphorylation. It also regulates the transcription of C/EBP isoforms. Variation of EIF4α2 might interact with other susceptibility genes in response to environmental and metabolic stress at the pancreatic level.

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**Altered functional variants of TGFβ-inducible transcription factor KLF11 are associated with diabetes**

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**Background and aims:** The role of TGFβ signalling in both pancreatic cell differentiation and growth suggest that an altered function of proteins within this pathway may contribute to diabetes. The pancreas enriched, TGFβ-inducible KLF11 (also known as TIEG2) has recently elicited significant attention because it participates in morphogenetic pathways in exocrine pancreatic cells. It is also expressed in endocrine islet cells, but its role in maintaining glucose homeostasis remains to be established. In this study, we analysed KLF11 as candidate diabetes susceptibility gene.
**Materials and methods:** We screened the KLF11 gene localized at chr2p25, for variants in 19 probands of French MODYx families (unlinked to any known MODY gene), 171 early onset (<40y) and 14 late onset (>45y) type 2 diabetic (T2D) probands with at least one affected first-degree relative. We constructed a linkage disequilibrium map for frequent polymorphic KLF11 variants (SNPs) in a pilot study of 96 control subjects and 66 probands from families that contributed to T2D linkage on chromosome 2 in the previously reported French genomewide scan. Thereafter, we analysed eight SNPs with a suggestive T2D association in our case control study of 313 T2D and 313 normoglyceamic subjects. By transfection experiments in CHO and β-cell lines with heterologous and other promoter constructs, we assessed the possible effects of the strongly associated variants on the transcriptional activity of KLF11.
**Results:** Two rare missense mutants and one frequent non-synonymous SNP showed association with diabetes in the French Caucasian population; 1) Mutant [+1039 G>T (Ala347Ser)] was found in a four generations MODYx family and was transmitted with diabetes/glucose intolerance in the three analysed generations. 2) Mutant [+659 C>T (Thr220Met)] co-segregated with diabetes in two early onset T2D families. 3) SNP Gln62Arg significantly associated with late onset T2D ($p=0.0001$). An "at risk" haplotype with Gln62Arg and three additional SNPs could be defined with an Odds ratio of 2.1 ($p=0.0002$ ; 95% confidence interval 1.4-3.2) and attributed risk of 36%. An altered 62Arg-KLF11 function was indicated by association of the "at risk" allele with a decreased insulin secretion in normoglycaemic subjects ($p=0.03$, for differences between the area under the curve of a standard OGTT). In vitro, all three KLF11 variants significantly altered the transcriptional activity of KLF11 protein. Moreover, transfection of the variants in pancreatic beta-cell lines showed a striking interference with transcriptional regulation of endocrine marker genes like PDX-1 and insulin.
**Conclusion:** Both the mutations occurring in MODY/early-onset T2D and the SNP associated with late-onset T2D evoke alterations in KLF11 function that may impair physiological processes either leading to monogenic diabetes (MODY) or contributing to polygenic T2D. Interestingly, KLF11 is at the cross-road of diabetes and cancer, since KLF11 is strongly repressed in pancreatic tumours. Our results suggest an important role of KLF11 in β-cell function and implicate for the first time the TGFβ-KLF11 transcriptional pathway in susceptibility to diabetes.
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**Impact of age and heritability on the control of muscle glycogen synthase activation in twins.**

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**Background and aims:** Insulin resistance in muscle tissue represents a major defect in type 2 diabetes (T2D). Storage of glucose as glycogen accounts for the quantitatively largest proportion of muscle glucose metabolism during insulin infusion in normal subjects, and represents a major defect of muscle glucose metabolism in insulin resistant states. Studies in first degree relatives have indicated a genetic origin of defective insulin activation of muscle glycogen synthase (GS) in T2D. The aim of this study was to evaluate the relative impact of genetic versus non- genetic factors on muscle GS activation and control in young and elder twins.
**Materials and methods:** A total of 184 twins in two age groups (22–31 and 57–66 yrs) underwent a 2-hour euglycaemic, hyperinsulinaemic clamp (40 mU/m2/min) combined with indirect calorimetry and excision of muscle biopsies during the basal and insulin stimulated state. The heritability was expressed as twice the difference of the interclass correlation of MZ and DZ twins ($h^2=2(r_{MZ}-r_{DZ})$).
**Results:** Elder twins had a significantly lower GS total activity compared to the younger twins in both steady state periods. Furthermore, the degree of GS phosphorylation at site 3 was significantly reduced in the elder twins. Glycogen content, GS fractional activity and GSK3 activity were similar in the two age groups. GS fractional activity increased and GS phosphorylation and activity of GSK3 decreased significantly during insulin stimulation in both young and elder twins. In younger twins glucose disposal (Rd) and non-oxidative glucose metabolism (NOGM) correlated significantly

with GS total activity (Rd: r=0.29, p<0.01; NOGM: r=0.29, p<0.01), GS fractional activity (Rd: r=0.45, p<0.00001; NOGM: r=0.48, p<0.00001) and GS phosphorylation (Rd: r=-0.22, p<0.03; NOGM: r=-0.20, p<0.05) during the clamp period. The increment in Rd and NOGM (delta values) correlated significantly with the increment in GS fractional activity (Rd: r=0.44, p<0.00001; NOGM: r=0.43, p<0.00001) and GS phosphorylation (Rd: r=-0.21, p=0.04; NOGM: r=-0.23, p=0.02). In elderly twins the increment in Rd and NOGM correlated significantly with the increment in GS fractional activity (Rd: r=0.39, p<0.001; NOGM: r=0.37, p<0.001). Similar interclass correlations were seen in elder MZ and DZ twins for glycogen, total and fractional GS activity and GSK3 activity during both steady state periods. There was a significant difference in interclass correlations between MZ and DZ twins for GS phosphorylation during both the basal (MZ: 0.52 vs. DZ: -0.05, p=0.02) and insulin stimulated states (MZ: 0.74 vs. DZ: 0.05, p=0.0001), indicating a genetic component. In the younger twins, glycogen, GS total and fractional activity and GS phosphorylation appeared to have a major environmental component A genetic influence was seen on GSK3 activity during the basal period due to the significant difference in interclass correlation between MZ and DZ twins (MZ: 0.63 vs. DZ: 0.17, p=0.008).

**Conclusion:** We demonstrate a major environmental component on GS activity per se in young and elder twins, which may, to some extent, question the view of a genetic defect in this enzyme per se underlying the pathophysiology of T2D. Interestingly, insulin activation of GS was intact with aging despite a highly significant reduction of NOGM and increase in body fat with age.

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**Impaired insulin secretion in pancreatic islets from congenic rats within the major glucose-controlling locus in the GK rat**

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**Background and aims:** The GK rat is a well-characterized animal model for spontaneous type-2-diabetes and several genome-wide significant loci for diabetes-associated phenotypes have been identified. We have previously demonstrated that the major glucose-controlling locus in F2-intercrosses between GK and F344 (29% of the genetic variance) is the *Niddm1* locus on chromosome 1. *Niddm1* is linked to reduced insulin secretion as well as insulin resistance in target tissues. Using congenic technology, the *Niddm1i* locus was identified (corresponding to 14 cM of the original 52 cM-*Niddm1* locus) that encodes hyperglycaemia combined with insulin deficiency. In an attempt to further narrow the locus and identify genes for defective insulin secretion in the GK-rat, we have established subcongenic rat strains carrying different parts of the *Niddm1i* GK-haplotype and characterized their capacity for insulin secretion.

**Materials and methods:** Transfer of GK alleles onto the F344 genome by repeated backcrossing generated the congenic strain NIDDM1I and five subcongenics within *Niddm1i*. F344 rats were used as a healthy control. *In vivo* glucose tolerance was investigated by an intraperitoneal glucose tolerance test (2.0 g glucose/kg bodyweight) in dexamethazone-treated (0.5 mg/kg bodyweight 36 and 8 h prior to glucose challenge) 95 days old rats. *In vitro* pancreatic hormone release was assayed in 60-min static batch incubations of ten islets (in triplicates) from rats sacrificed at day 60. The control incubation medium contained 3.3 mM glucose, to which glucose (final conc. 20 mM), the mitochondrial substrate alpha-KIC (20 mM), glibenclamide (20 µM), or high $K^+$ (50 mM), were added. Insulin and glucagon levels were assayed by RIA.

**Results:** *In vivo* glucose tolerance tests did not reveal any significant differences between the dexamethazone-treated congenic strains. *In vitro*, glucose stimulated insulin release 3-fold in healthy F344 (5.2 ± 1.1 and 18.0 ± 5.7 ng/islet/h, at 3.3 and 20 mM glucose, P=0.09). Glucose-evoked insulin release was reduced by 75% in rats carrying the GK haplotype for the entire *Niddm1i* locus (P=0.01 vs. F344), as well as in subcongenic strains N1IREC6 (63% reduction, P=0.009 vs. F344) and N1IREC11 (58% reduction, P=0.02 vs. F344), which all share a 3.5 cM region of the GK genome containing ~10 genes. In these congenic strains, defective insulin secretion was not only observed in response to glucose stimulation. In fact, insulin release was significantly reduced in response to all stimuli (alpha-KIC, glibenclamide or high $K^+$). By contrast, glucagon secretion was unaffected in islets from all strains.

**Conclusion:** The impaired insulin secretion in NIDDM1I and two of the subcongenic strains suggest that the secretory defect is not caused by defective glucose metabolism but occur in a later stage of the insulin secretion process such as, e. g., vesicle translocation or priming of vesicles to the membrane, or the number of functional beta cells. A 3.5 cM region on rat chromosome 1 containing ~10 genes is a major susceptibility locus for defective insulin secretion in diabetic GK rats. However, contribution of other susceptibility loci are required for the development of a diabetic phenotype.

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**Variations in *PPARγ* and *APM1* genes predict cardiovascular mortality in Type 2 diabetes patients**

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**Background and aims:** Type 2 diabetes [T2D] and features of the metabolic syndrome [MSDR] are severe risk factors for cardiovascular morbidity and mortality. Polymorphisms in the *PPARγ*, adiponectin [*APM1*] and calpain 10 [*CAPN10*] genes have been associated with T2D and features of MSDR in several different populations and both *PPARγ* and adiponectin have been reported to have anti-atherogenic properties. Aim of this study was to investigate whether polymorphisms in *PPARγ*, *APM1* or *CAPN10* can predict cardiovascular mortality in T2D patients.

**Materials and methods:** A total of 1309 T2D patients aged >35 years (606 males, 703 females, age 67.2 ± 11.7 years, BMI 28.3 ± 4.7 kg/m$^2$, 72.6% with MSDR defined according to WHO99 guidelines, 68.4% with hypertension and 15.3% with microalbuminuria) and participating the large Botnia T2D family study were genotyped using single base pair extension method on ABI3100 (*CAPN10* SNPs 43 and 44), allelic discrimination on ABI7900 (*APM1* SNPs -4041, 276 and 2019) or PCR-RFLP (*PPARγ* Pro12Ala). Expected risk-genotypes were defined according to earlier reported T2D association study results. Mortality was assessed with a median follow up of 7.4 years and obtained from central death-certificate registry. Cardiovascular mortality was classified using the International Classification of Diseases. For mortality analyses we used Cox regression analyses with robust variance estimate, adjusted for sex and family correlations.

**Results:** During the follow-up period, 463 individuals (33.3%) had died and of them 265 (20.2%) due to cardiovascular disease (123 males, 142 females, age 75.2 ± 8.8 years, BMI 27.7 ± 4.7 kg/m$^2$, 71.7% with MSDR, 74.0% with hypertension and 31.0% with microalbuminuria). Male sex (p=0.00020), smoking (p=0.0036), fasting serum insulin (p=0.031) and microalbuminuria (p<0.00001) were significant predictors of cardiovascular mortality among the T2D patients. Of the genetic factors, *APM1* SNP276 (G-allele), SNP2019 (II-genotype) and *PPARγ* Pro12Ala (ProPro-genotype) were found to be significant predictors of cardiovascular mortality (hazard ratio 1.87 [1.11-3.15], p=0.019; 1.42 [1.10–1.82], p=0.0073 and 1.46 [1.08-1.98], p=0.014, respectively). *CAPN10* polymorphisms were not significantly associated with cardiovascular mortality. None of the studied genes were associated with mortality due to other than cardiovascular causes.

**Conclusion:** Genetic variations in T2D susceptibility genes *PPARγ* and *APM1* predict cardiovascular mortality in T2D patients.

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**Multiple subtypes of diabetes are associated with activating mutations in *KCNJ11*, which encodes the Kir6.2 sub-unit of the beta-cell ATP sensitive potassium ($K_{ATP}$) channel**

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**Background and aims:** Recently we have shown that permanent neonatal diabetes (PNDM) can be caused by heterozygous activating mutations in *KCNJ11* that encodes the Kir6.2 subunit of the β-cell ATP sensitive potassium ($K_{ATP}$) channel. These mutations are a common (~34%) cause of PNDM. Common genetic variation in *KCNJ11* (E23K) has been shown to predispose to type 2 diabetes (T2DM). We hypothesised that an array of naturally occurring *KCNJ11* activating mutations could exist with a range of severities resulting in diabetes with a spectrum of clinical presentations.

Therefore we investigated the role of *KCNJ11* mutations in transient neonatal diabetes (TNDM) and type 1 diabetes (T1DM) diagnosed under the age of 2 yrs.
**Materials and methods:** *KCNJ11* was sequenced in 11 probands with TNDM in which chromosome 6q24 abnormalities had been excluded and in 77 subjects with T1DM.
**Results:** Novel heterozygous *KCNJ11* mutations (G53S, G53R, I182V), which co-segregated with diabetes, were identified in 3 TNDM probands. In the T1DM cohort we identified 2 subjects with heterozygous mutations; one previously reported (R201C) and one novel (R176C). None of the mutations were identified in 100 normal chromosomes. All subjects were pancreatic auto-antibody negative and the T1DM mutation positive patients did not have high risk predisposing HLA genotypes. Including our PNDM data the range of age of presentation of diabetes for subjects with *KCNJ11* mutations is from birth to 17 months.
**Conclusion:** Our study demonstrates that in addition to predisposing to T2DM *KCNJ11* mutations can cause diabetes that presents as PNDM, TNDM and T1DM. The identification of a *KCNJ11* mutation could have implications for treatment as patients may be treated by oral sulphonylureas.
*This study was funded in Exeter by the Wellcome Trust and Diabetes UK*

# OP 37
# Hypoglycaemia

## 217

**Differential effects of glucose and aminoacids on counterregulatory responses to insulin-induced postprandial hypoglycemia**
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**Background:** It has been shown that counterregulatory (CR) hormone responses to insulin-induced hypoglycemia (I-IH) are greater both in non-diabetic (N-DS) and T1 DM subjects in the postprandial (mixed meal) as compared to the fasting state.
**Participants and methods:** To establish the relative role of macronutrients such as carbohydrate and proteins on CR responses we studied 7 N-DS (M/F 3/4, 32 ± 3.5 years old, BMI 22 ± 1) during clamped I-IH (2.5 mmol/l for 40 min) under three conditions: 1) placebo (P), 2) a mixture of aminoacids 60g (AA), 3) glucose 28g (G), each treatment given orally 90 min before clamped I-IH. Plasma glucose (PG) and insulin concentrations were not different ($p>0.2$) under the three conditions.
**Results:** Plasma CR hormones increased during clamped I-IH under all three conditions, however glucagon levels increased more after AA ingestion (Mean±SE, A:306 ± 18, G:142 ± 9.0, P:100 ± 5.0 pg/ml, $p<0.001$), GH levels tended to be higher in G than A and P (A:6.1 ± 1.8, G:15.4 ± 4.2, P:5.2 ± 1.5 ng/dl, $p=0.06$), adrenaline and noradrenaline were not different. Overall glucose infusion rates were lower in AA and G than in P (2.6 ± 0.7, 2.4 ± 0.6, 5.4 ± 0.8 mg/kg/min, respectively, $p<0.01$). Plasma C-peptide levels were less suppressed in AA and G than in P (A:0.5 ± 0.13, G:0.6 ± 0.16, P:0.2 ± 0.08 nmol/l, $p<0.01$). Plasma AA concentration increased to the range observed after mixed meal (AA:2.6 ± 0.16, G:2.0 ± 0.14, P:1.9 ± 0.08 mmol/l, $p=0.021$). Total symptoms scores were not different. Cognitive tests which were sensitive to hypoglycemia (Trail-Making B, Digit Span Backward) deteriorated less in AA than G and P (A:-2.6 ± 0.7, G:-9.6 ± 1, P:−11.6 ± 1, composite Z-scores, $p<0.02$).
**Conclusions:** We conclude that oral AA more than G potentiates glucagon responses vs P similarly to mixed meal. The less deterioration of cognitive tests after oral AA during I-IH may be compatible with its use by the brain

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**The effect of hypoglycaemia unawareness on brain glucose content, transport and metabolism during euglycaemia and hypoglycaemia in man**
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**Background and aims:** In Type 1 diabetes, repeated exposure to hypoglycaemia induces impairment of the protective neurohumoral counterregulatory responses to impending hypoglycaemia, resulting in hypoglycaemia unawareness. The mechanism is thought to be a failure of brain glucose sensing. Our hypothesis was that brain glucose transport is upregulated in hypoglycaemia unawareness, to maintain brain glucose metabolism in the cerebral glucose sensor.
**Materials and methods:** 12 men with Type 1 diabetes, 6 of whom were aware and 6 unaware of hypoglycaemia, were recruited. Hypoglycaemia unawareness was defined by a history of severe hypoglycaemia requiring third party assistance and home blood glucose records showing more than 3 episodes of asymptomatic biochemical hypoglycaemia (less than 3.5 mmol/l) over 2 weeks. Each subject was studied on 2 occasions, in random order, with an 11C-labelled 3-0-methyl glucose tracer positron emission tomography scan, once during euglycaemia (5 mmol/l) and once during hypoglycaemia (2.6 mmol/l). Brain intracellular glucose concentration (content); rate constants for glucose transport (transport); and indirectly glucose phosphorylation (metabolism) were measured.
**Results:** During hypoglycaemia, only the aware group experienced symptoms and correctly identified hypoglycaemia. The adrenaline response to hypoglycaemia was greater in the aware group (28.4 vs 16.5 nmol/l/50 mins, $p< 0.05$). Whole brain glucose content was not different at euglycaemia between groups and fell significantly in both the aware (1.18 ± 0.45 to 0.02 ± 0.2 mM, $p<0.01$) and unaware during hypoglycaemia (1.07 ± 0.46 to 0.19 ± 0.23 mM, $p<0.01$), with no significant difference between groups. Whole brain glucose metabolism was not different between groups at euglycaemia but was lower at hypoglycaemia in the unaware (aware 13.95 ± 2.37,

unaware 10.16 ± 0.8 μmol/100g /min), p<0.05). Brain glucose transport was no different at euglycaemia compared to hypoglycaemia and no differences were seen between groups. Regional analysis with statistical parametric mapping highlighted Brodmann area 10, an area concerned with memory function. Quantitative analysis in this area showed a reduced fall in glucose content compared to whole in both groups with no differences in transport. Glucose metabolic rate in this area fell during hypoglycaemia in the unaware group only (12.9 ± 3.7 to 7.8 ± 1.9 μ/100g/min, p< 0.05).
**Conclusions:** There is no global or regional upregulation of brain glucose transport to explain the syndrome of hypoglycaemia unawareness in type 1 diabetes. Whole brain glucose content falls similarly in the aware and unaware during hypoglycaemia and there is no preservation of brain glucose metabolism in the unaware. The significant fall in glucose metabolic rate during hypoglycaemia in Brodmann area 10 in the unaware may explain the memory problems reported by many patients with reduced hypoglycaemia awareness.
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## 219

### The epidemiology of hypoglycaemia in a UK population with Type 2 diabetes

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**Background and aims:** The epidemiology of the manifestations of hypoglycaemia among patients with diabetes is not well understood. The aim of this study was to evaluate the epidemiology and impact of hypoglycaemia in a UK population with type 2 diabetes (T2DM).
**Materials and methods:** A postal survey mailed to 3500 subjects with diabetes identified through hospital review, included questions on: the frequency and impact of hypoglycaemia; diabetes management; lifestyle; the EQ-5D and diabetes-related complications. Self-reported hypoglycaemia was classed as moderate, nocturnal and severe. Detailed phenotypic details were available through the Health Outcomes Data repository (HODaR).
**Results:** At this point, 729 patients responded to the survey (20.8%). Of the respondents, 62.3% had Type 2 diabetes and 59.0% were male. The mean age was 67.1 and 66.4 for males and females, respectively. The overall mean frequency of hypoglycaemia was 10.9 events per person per year (0.9 severe, 6.6 moderate, 3.3 nocturnal). Of these subjects, 35.9% had no reported hypo's. In people who experienced a severe hypoglycaemic event, 55.6% reduced their insulin dose to prevent future hypoglycaemic events. Other preventative behaviour included eating more (75%), reducing physical exercise (91.7%) and taking sick leave (50%). On average, it took 8.6 days and 2.8 days to recover fully from a severe and a nocturnal hypoglycaemic event, respectively. The rate of moderate, nocturnal and severe hypoglycaemia by treatment category was 21.7 per person year (PPY) on insulin in any combination, 8.9 PPY on only biguianides, 9.3 PPY on only sulphonylureas, 7.6 PPY on diet only.
**Conclusion:** Hypoglycaemia-like symptoms were a surprisingly common manifestation of T2DM, although that this may be due largely to a lack of understanding of hypoglycaemia as an adverse event in non-insulin treated patients. Nevertheless, measures should be taken to reduce this affect whilst maintaining good glycaemic control.

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### Frequency and predictors of severe hypoglycaemia in insulin-treated Type 2 diabetes

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**Background and Aims:** Risk of severe hypoglycaemia (SH) in insulin-treated type 2 diabetes is reported with highly variable rates and only few studies have assessed the influence of potential risk factors on occurrence of SH. The aim of the study was to assess the incidence of SH and to identify risk factors for SH in a large cohort of patients with insulin-treated type 2 diabetes.
**Materials and Methods:** 401 patients with insulin-treated type 2 diabetes with age 66 (39–89) years [median (range)], diabetes duration 15 (2–39) years, duration of insulin therapy 7 (1–28), C-peptide 628 (10-4146) pmol/L, $HbA_{1c}$ 8.3 (5.3–12.3)%, completed a questionnaire about hypoglycaemia and hypoglycaemia awareness (HA) (scored on a 4-point scale). Primary endpoint was number of SH (defined as episodes needing help from other persons, expressed as episodes/patient-year). To estimate predictors of SH a zero-inflated Poisson regression model was used for analysis. This model takes into account excess zero counts in observing SH, and presumes that each participant has probability of belonging to a class, that is immune to SH, or a class who is at risk of such events.
**Results:** In the preceding year (data collection completed August 2003) the total number of SH was 178, corresponding to an incidence rate of 0.44 episodes/person-year. A total of 66 patients experienced at least one event, corresponding to a prevalence of 16.5%. The distribution of SH was highly skewed. Self-estimated awareness of hypoglycaemia was impaired in 46% of the patients. Risk factors for susceptibility to SH (387 included in analysis) were impaired awareness [odds ratio (OR) 2.9; 95% confidence interval (CI) 1.6-5.2], long duration of insulin therapy (OR 2.1/ten years; CI 1.1-3.9), and presence of neuropathy (OR 2.3; CI 1.2-4.2). For the number of episodes of SH encountered, shorter diabetes duration [rate ratio (RR) 2.0/ten years; (CI) 1.4-2.5], and alcohol intake above 2 units/day (RR 2.5; CI 1.4-5.0) were predictors of SH.
**Conclusion:** In insulin-treated type 2 diabetes the incidence of SH is higher than reported by most studies. Impaired awareness and long duration of insulin therapy are associated with risk of SH as in type 1 diabetes.

# OP 38

# Mood disorders in diabetes

## 221

**How adequate is the treatment for depression in diabetic patients?**

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**Background and aims:** Depression is a prevalent co-morbid condition in diabetes. This study was aimed at determining the occurrence of depression in type 2 diabetic patients, as well as the degree to which depression was adequately treated.
**Materials and methods:** A random sample of 405 type 2 diabetic outpatients were screened for depression with the Center for Epidemiological Studies-Depression (CES-D) instrument. Patients with CES-D scores ≥16 were invited for a psychiatric interview relying on Axis I disorders of the DSM-IV (SCID) to establish the clinical significance of their symptoms. Psychological anamnestic data and data on current life circumstances were collected by a semi-structured interview. Adequate treatment of depression was defined as the use of either an antidepressant or mood stabilizer for a minimum of 30 days, or at least 8 outpatient visits with any professional qualified in psychotherapy.
**Results:** Of the examined patients 24% had CES-D scores ≥16 (n=96, 66% female, aged 57 ± 8 years, educated for 10 ± 3 years, having diabetes for 9 ± 6 years, treated with insulin in 44% of cases, with BMI 29 ± 4 kg/m$^2$ and $HbA_{1c}$ 7.9%± 1.6%), and in 36% of them clinical depression was confirmed by SCID (64% female, aged 55 ± 8 years, with 10 ± 3 years of education, having diabetes for 9 ± 6 years, 43% insulin treated, with BMI 29 ± 4 kg/m$^2$ and $HbA_{1c}$ 7.9%± 2.2%). When compared with subjects free of severe depressive symptoms (CES-D<16) and those with mild depression (CES-D ≥16 but not confirmed by a diagnosis of depression), clinically depressed patients were found to be taking psychotropic medication in a significantly greater proportion (71% vs. 47% in the group with mild depression and 27% in the group free of severe depressive symptoms $\chi^2$=27.72; p<0.005). However, only 22% of patients with clinical diagnosis of depression were treated for depression in accordance with the established criteria, while the remaining majority was recommended drugs that could not be supposed to specifically relieve depressive symptoms. Thirty-two percent were without any treatment, among them one diagnosed with major depression during the screening procedure.
**Conclusion:** The obtained data indicate that depression as a co-morbid condition in diabetes is neither sufficiently recognized nor specifically treated in the majority of patients affected.

## 222

**Disordered eating behaviors among adolescents with Type 1 diabetes**

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**Background and aims:** The most vulnerable time to develop an abnormal eating attitude is adolescence. The existence of eating disorders in adolescents with type 1 diabetes could disrupt glycemic control and increase the risk of long-term complications. The aim of the study was to assess diabetes specific behavior related to eating attitudes and determine if diabetes management could explain development of eating disorders.
**Materials and methods:** We assessed eating attitudes by using the Eating Attitudes Test (EAT-26) in 41 adolescents with type 1 diabetes, aged 12–17 years. The responses were compared with those of a nondiabetic, control group of 50 adolescents - similar age and sex distribution. EAT-26 consists of questions grouped according to three features: dieting, bulimia and food preoccupations, and oral control. Glycemic control was assessed by glycated hemoglobin (HbA1 c) assay. The mean value for the last 12 months was considered.
**Results:** The mean EAT – 26 scores in the diabetic group (19.26 ± 8.97 ) was higher than in the control group (9.38 ± 7.74), particularly in girls aged 13–15. Three risk categories based on the EAT scores were proposed: no risk – EAT 0–9 (12.2% diabetes group vs. 68% control), low risk - EAT 10–19 (53.6% diabetes group vs. 20% control), high risk – EAT ≥20 (34.2% diabetes group vs. 12% control). Higher scores on the diet subscale of EAT (71.4%) but lower scores on the bulimia (9.34%) and oral control (19.26%) subscales were found in diabetic patients. We noticed a cut-off value of 15 for EAT – score regarding metabolic control, assessed by HbA1 c: EAT scores under 15 were associated with lower HbA1 c values (8.93 ± 1.6%) than those over 15 (9.43 ± 1.5%) (P=0.43). Unexpectedly, the EAT high-risk diabetic patients didn't report an increased incidence of ketoacidosis. There were no differences regarding the values of BMI between the two groups, despite the fact that diabetic girls were slightly heavier (22.17 ± 2.6 kg/m$^2$) than the control girls (18.59 ± 1.51 kg/m$^2$). 9 diabetic patients (9%) reported binge eating. This happened when they felt hypoglycemia.
**Conclusion:** Adolescents with type 1 diabetes reported more disordered eating attitudes than the control group. Features associated with type 1 diabetes and its treatment, such as food preoccupation, dietary restraint and weight gain could explain the higher diet subscale score. Routine evaluation of young type 1 diabetics (especially girls) with poor glycemic control could help physicians in the management of diabetes by identifying patients prone to abnormal eating attitudes. Disordered eating patterns in adolescents with type 1 diabetes are likely to compromise their glycemic control.

## 223

**Developmental, behavioural and health factors related to depression in Type 1 diabetes mellitus sufferers and healthy controls**

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**Background and aims:** Type 1 diabetes presents considerable adaptive challenges for sufferers. However, little is known of the processes by which psychological and physiological adaptation to the challenges of Type 1 diabetes are managed during adolescence and young adulthood. The present study examined the role of developmental, behavioural and health factors in the psychological adjustment to Type 1 diabetes. Specifically, factors related to depression among Type 1 and healthy controls adolescents and young adults were investigated.
**Materials and methods:** A sample of 123 participants with Type 1 diabetes and 123 healthy controls, aged 12-26 years, were recruited from several states of Australia, and completed a battery of self-report questionnaires. Participants were 50% adolescents, and 67.1% females. The average duration of Type 1 diabetes was 7.71 years.
**Results:** Using independent t-test and discriminant function analyses, findings revealed that young adults with Type 1 diabetes reported significantly higher depression scores ($M$ = 1.81, $SD$ = 1.32) than healthy controls ($M$ = 1.35, $SD$ = 1.16), $t$ (118) = 2.046, $F$ (1, 111) = 4.02, $p < .05$. They also exhibited lower levels of problem focused coping ($M$ = 30.00, $SD$ = 10.95) than the young ones without diabetes ($M$ = 34.22, $SD$ = 9.65), $t$ (119) = 2.245, $F$ (1, 111) = 5.35, $p < .05$. For the adolescent group, diabetes sufferers reported more health behaviours ($M$ = 49.03, $SD$ = 7.71) than healthy controls ($M$ = 45.02, $SD$ = 6.51), $t$ (123) = 3.153, $F$ (1, 108) = 10.76, $p < .01$, but there were no differences between the young adult diabetes sufferers (M = 45.97, SD = 7.65) and the young adult healthy controls (M = 44.46, SD = 8.00), t (119) = 1.062, $p > .05$. Development-independent living significantly differentiated diabetes sufferers ($M$ = 5.68, $SD$ = 1.90) from healthy controls ($M$ = 6.16, $SD$ = (1.74), $t$ (242) = -2.039, $p < .05$. Furthermore, discriminant function loadings in excess of .30 indicated that development-independent-living, anxiety, and social support were good predictors for distinguishing between young adult diabetes sufferers and young adult healthy controls. According to this criterion, young adults with Type 1 diabetes exhibited less independent living skills ($M$ = 6.52, $SD$ = 1.72), more anxiety symptoms ($M$ = 41.49, $SD$ = 11.01), had more social supports ($M$ = 24.97, $SD$ = 11.87) and more satisfaction from these supports ($M$ = 0.73, $SD$ = 0.36) than young adult healthy controls ($M$ = 7.02, $SD$ = 1.47 for development-independent living; $M$ = 38.54, $SD$ = 9.56 for anxiety; $M$ = 21.12, $SD$ = 11.02 for social support: number of supports; $M$ = 0.64, $SD$ = 0.35 for social support: degree of satisfaction). Using multiple regression analyses, findings showed that development and presence of disease were significant predictors of depression, $F$ (11, 234) = 12.46, $p < .01$.
**Conclusion:** Interventions aimed at the development of independent living and problem-focused coping skills could be important in the management of depression in Type 1 diabetes sufferers, particularly for young adult sufferers.

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**Gender differences in hardiness, degree of depression and anxiety in patients with diabetes**

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**Background and aims:** We sought to determine the degree of hardiness, degree of depression, anxiety and anxiousness in patients with diabetes attending an educational course while hospitalized.
**Materials and methods:** The patients with diabetes were examined, using the PVS questionnaire determining the degree of hardiness (resistance to stress) (Kobasa), Spielberger's State-Trait Anxiety Inventory and Beck's Depression Inventory. The hardiness questionnaire produces an overall score and three scores from individual subscales: commitment, control and challenge. The sample included 95 men (44.6%) and 118 women (55.4%), with a mean age of 40.6 ± 15 years; Type-1 diabetes mellitus (DM) was diagnosed in 151 individuals (71%) while Type-2 DM was present in 62 individuals (29%), mean duration of disease was 10.6 ± 8.3 years; the most frequent therapy was intensified insulin regime (83%), two insulin doses (7.6%), PAD (4.3%) and the insulin pump (3.3%). Mean metabolic disease control (HbA1c) was 9.6% ± 2.4% in Type-1 DM, and 9.7% ± 1.7% in Type-2 DM. Very unsatisfactory diabetes control-a mean HbA1c higher than 9%-was seen in 62.6% of patients while a HbA1c over 10% was present in 41.4% of patients with diabetes.
**Results:** 1. Highly significant differences were demonstrated between male and female patients in the degree of anxiousness ($p<0.001$), current anxiety ($p<0.001$) and depression ($p<0.05$). 2. The score of depression, current anxiety and, even more so anxiousness, was significantly higher in women with diabetes compared with men with diabetes; the inter-individual differences were bigger in women than in men. 3. The mean values of current anxiety and anxiousness were higher in patients with DM than the population means. 4. The elevated scores on Beck's Depression Inventory in our patients suggest presence of depressive symptoms. 5. The degree of hardiness correlates inversely with disease duration; the longer the duration of diabetes, the lower the patient's hardiness. 6. No significant differences were found between the sexes in the degree of hardiness. 7. No significant differences were demonstrated in the degrees of anxiety, anxiousness, and depression between our men and women as related to the type of diabetes mellitus.
**Conclusion:** Results show that the impact of diabetes on psychological well-being is more marked in women. Women with diabetes tended to experience significantly higher psychological discomfort (with more manifestations of depression, with more concern, fear, and anxiety) than men. There was no difference between women and men of our group in terms of age, diabetes duration, and hardiness. Our conclusions highlight a different psychological gender-dependent context of diabetes. These circumstances should ideally be considered in education and overall therapeutic approach to women.
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# OP 39

# Macrovascular disease: cellular mechanism on animals

## 225

**The platelet derived growth factor-beta receptor and beta-3 integrin are differentially influenced by cyclic stretch in porcine vascular smooth muscle cells, cultured in high glucose.**

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**Background and aims:** Hyperglycaemia and hypertension are associated with accelerated atherosclerosis. We have shown previously that the platelet derived growth factor-beta (PDGF-beta) receptor and beta-3 integrin levels are increased by high glucose (25 mmol/l; HG) or by cyclic stretch (flexion) in vascular smooth muscle cells (VSMC). The aim of this study was to determine the combined effects of glucose and 'hypertensive' levels of stretch on PDGF-beta receptors and beta-3 integrin in VSMC.
**Materials and methods:** Primary, thoracic aorta-derived porcine VSMC, were plated on surfaces coated with surrogate basement membrane (Matrigel) and exposed to normal glucose (5 mmol/l; NG) or HG and/or cyclic stretch (60 cycles per min, 24 h) within physiological and 'hypertensive' ranges using the Flexercell apparatus. Protein levels were analysed by immunoprecipitation, followed by Western blotting. All results are expressed as a percentage of NG unflexed (100%).
**Results:** HG increased both PDGF-beta receptors and beta-3 integrin (+168%, $p<0.01$; and +48%, $p<0.05$ respectively) in static incubations. MEK1/2 inhibitor (PD98059, 10 µmol/l) reduced the HG effect on receptor and beta-3 integrin levels ($p<0.05$).
'Physiological' flexion (0–3%) increased ($p<0.01$) receptor levels in both NG (+92%) and HG (+149%). 'Hypertensive' stretch (9-12%) had no further effect. The MEK1/2 inhibitor reduced ($p<0.05$) the increases induced by both levels of flexion only in the presence of HG. In the presence of NG and HG conditions, the p38 MAPK inhibitor (SB203508, 600 nmol/l) reduced the receptor response to 0–3% and 9-12% flexion ($p<0.05$).
In contrast the beta-3 integrin was increased ($p<0.01$) by 0–3% flexion in the presence of NG or HG conditions (NG: +47%, HG: +53%). At 'hypertensive' levels of flexion, the increase was additive in the presence of HG (+112%, $p<0.01$). The MEK1/2 inhibitor reduced ($p<0.05$) the increase induced by 0–3% flexion in NG and HG, but had less of an effect at 9–12% flexion.
Activated ERK1/2, a downstream target of MEK1/2, was maximally increased by HG and 0–3% flexion (+232%, $p<0.01$), whereas activated p38 levels were predominantly increased by HG and 9-12% flexion (+168%, $p<0.05$).
**Conclusion:** MAPK pathways are involved in the upregulation of the PDGF-beta receptor and beta-3 integrin by HG and cyclic stretch. However MAPK pathway signalling is differentially influenced by the level of stretch: the ERK1/2 pathway is activated during 'physiological' stretch whereas at 'hypertensive' levels of stretch, signalling is predominately by the p38 MAPK pathway.

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**High glucose and/or insulin increase the Ras Associated with Diabetes (RAD) expression in rat vascular smooth cells (vSMC): lack of these effects on vSMC from diabetic rats**

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**Background and aims:** Both hyperglycemia and hyperinsulinemia can cause vascular damage complications in diabetic patients, however the molecular mechanisms involved are not fully understood. Rad (Ras associated with diabetes) is a small G protein identified by subtraction cloning and found to be overexpressed in skeletal muscle of patients with type 2 diabetes mellitus. Rad overexpression has been observed in the vascular wall of diabetic animals: however, Rad trascriptional regulatory mechanisms are poorly characterized.
**Materials and methods:** In the present study, Rad expression was measured by Real Time PCR in cultures of vSMC obtained from aorta medial layer of 10 diabetic (90% pancreatectomy, DR) and 10 control (sham surgery, CR) rats, in the presence or in the absence of 25 mM glucose and/or 10 nM

insulin. In the same experimental conditions, we also evaluated the effect of LPS (20 μg/ml) on RAD expression.
**Results:** In CR vSMC, both high glucose and/or insulin were able to significantly increase Rad expression in a time-dependent manner. Following glucose and/or insulin stimulation, RAD mRNA levels started to increase at 30 min, peaked at 2 hrs, and returned to baseline after 6 hrs. LPS (20 μg/ml) significantly increased the stimulatory effect of both high glucose and/or insulin on CR vSMC. On the contrary, in DR vSMC, Rad was overexpressed as compared to CR vSMC in the basal state, but neither LPS, or insulin and/or high glucose stimulation significantly increased RAD mRNA.
**Conclusion:** The demonstration that glucose and/or insulin, in presense or absence of LPS, can modulate Rad transcription and they do so differently in cells from control and diabetic animals may provide new insight in the mechanisms linking diabetes and atherosclerosis.

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**The enhanced vasoconstriction in diabetes mellitus is mediated by reduced nitric oxide bioavailability**
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**Background and aims:** There is a lot of evidence that the vasoreactivity of vessels is altered by diabetes. From clinical and experimental studies follows that the endothelium dependent vasodilation is often impaired, but mostly vasocontractility is enhanced in diabetes, too. Neither the defect underlying the impairment of endothelial relaxation nor that causing the increased vasoconstriction has been clarified. It might be that both defects are not interdependent from each other but are a consequence of the reduced bioavailabilty of nitric oxide (NO) in diabetes which is not only determined by the activity of endothelial nitric oxide synthase (eNOS), but also by the activity of extracellular superoxide dismutase (ecSOD). ecSOD might have a large impact on bioavailability of NO since it removes reactive oxygen species (ROS) and thereby prevents inactivation of NO.
**Materials and methods:** To study vasoreactivity, rings of the thoracic aorta from mice and rats were incubated in an organ chamber and the reactions in response to acetylcholine (Ach, stimulator of NO) or phenylephrine (Phe) were observed. Diabetes was induced by streptozotocin. In addition aortae of mice were studied in which the gene encoding endothelial nitric oxide synthase (eNOS) was deleted (NOS-KO). ecSOD activity was determined by a biochemical colorimetric assay based on the oxidation of nitrotetrazolium blue using xanthin/xanthin oxidase as a radical inducing system.
**Results:** Vasocontraction in response to Phe was clearly increased by diabetes in wildtype mice (6,49 ± 1,04 mN vs. 12,68 ± 1,07 mN). A similar effect was observed when NOS was inhibited by L-NMMA (5,59 + 1,1 mN vs. 11,55 + 1,1 mN). In diabetes vasocontraction was increased by L-NMMA in addition to that already caused by diabetes. In NOS-KO mice maximal contraction was achieved with 21,2 + 1,5 mN in controls and 22,3 + 1,6 mN in diabetic animals (not significantly different). A graduated inhibition of NOS increased vasoconstriction in response to Phe in a comparable way, however the initial values were different between controls and diabetics. To study whether ecSOD plays a role for an alteration of bioavailability of NO, aortic rings from control and diabetic rats were pre-incubated with diethydithiocarbamat (DETC), an inhibitor of superoxide dismutases (SOD). Whereas in controls DETC only slightly inhibited the NO dependent vasodilation (max. relaxation 10,4 vs. 18,1% with DETC), DETC had a much larger impact on relaxation in diabetes (max. relaxation 8,7 vs. 32,7% with DETC). The activity of ecSOD was reduced for about 50% in diabetic vessels as compared to controls (1,337 ± 0,123 $U^*min^{-1}*100\ mg^{-1}ww$ vs. 2,583 ± 0,279 $U^*min^{-1}*100\ mg^{-1}ww$).
**Conclusion:** These data indicate that the enhanced vasoconstriction seen in diabetes is presumably caused by formation of ROS and a consequence of the reduced bioavailability of NO. Complete loss of NOS had comparable effects on vasoconstriction in diabetes and control animals. Partial inhibition of NOS by L-NMMA had similar effects on vessels from control and diabetic mice if the relative alterations are regarded. In the basal state vasoconstriction is enhanced in diabetes since a substantial amount of NO is obviously inactivated by ROS following from the inhibition of SOD by DETC. Whereas in controls the rate of ROS formation is low, diabetes accelerates the generation of ROS. This effect might be further aggravated by the diminution of ecSOD activity. Both effects would reduce the bioavailability of NO.

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**Inducible Nitric Oxide Synthase (iNOS) activity is increased in vascular smooth muscle cells (vSMC) from thoracic aorta of diabetic rats**
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**Background and aims:** Impaired vascular bioavailability of Nitric Oxide (NO) has been proposed among the mechanisms linking diabetes and atherosclerosis. We have previously shown that in vSMC obtained from thoracic aorta of diabetic rats (DR), eNOS mRNA levels and NOS activity are increased, as compared to Control Rats (CR) cells, while intracellular cGMP level is lower and superoxide anion production is significantly greater. The same cell cultures (DR) showed a proliferative phenotype as compared with the contractile phenotype shown by CR vSMC. These data indicate that in the vascular wall a diabetic milieu can induce an increased number of vSMC proliferative clones which persist in culture and are associated with increased eNOS expression and activity. However, iNOS expression and activity in vSMC obtained from diabetic rats have not been fully characterized.
**Materials and methods:** In this study, we measured iNOS expression and activity (by RT-PCR, Western Blot and conversion of 3H-arginine in 3H-citrulline, respectively) in cultures of vSMC obtained from aorta medial layer of 10 diabetic (90% pancreatectomy, DR) and 10 control (sham surgery, CR) rats, in the presence or in the absence of 20 μg/ml LPS and/or 10 nM insulin.
**Results:** After 24 hrs incubation, in both CR and DR vSMC, LPS similarly and significantly induced an iNOS mRNA and protein levels increase (68 ± 6 and 70 ± 5 Arbitrary Units CR and DR, respectively). Insulin (10 nM) significantly enhanced LPS stimulatory effects in both cell cultures (100 ± 8 vs 68 ± 6 Arbitrary Units CR, $p< 0.05$, 98 ± 7 vs 70 ± 5 Arbitrary Units DR, $p< 0.05$). In the same experimental conditions, iNOS activity was significantly greater in DR vSMC as compared to CR cells (about 7 fold increase DR vs CR, $p<0.05$). Insulin enhanced iNOS activity significantly more in DR than in CR cells (by 1.8 and 1.3 folds respectively, both $p<0.05$). However, notwithstanding increased iNOS activity, cGMP levels were not different between DR and CR cells either in the basal or in the insulin stimulated state.
**Conclusion:** Since, as we have previously shown, DR cells in these culture conditions exhibit a marked increase in $O2^-$ production, it is tempting to hypothesize that in DR cells the proliferative phenotype is associated with increased iNOS activity and hence increased NO generation, but the concomitant presence of a redox imbalance responsible for quenching and/or trapping of NO leads to reduced NO availability and impaired NO biological effects.

# OP 40

# Diabetic nephropathy: genetics and mechanisms

## 229

**Impact of left ventricular hypertrophy on the prognosis in patients with Type 2 diabetes and nephropathy. Insights from the RENAAL study**

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**Background and aims:** As the role of left ventricular hypertrophy (LVH) as an independent risk factor in patients with diabetic nephropathy (DN) is not clear, the present posthoc analysis of the RENAAL (Reduction of Endpoints in NIDDM with the Angiotensin II antagonist Losartan) study was undertaken to explore the impact of LVH on cardiovascular (CV) and renal events in type 2 diabetic patients with DN in the absence or presence of losartan treatment.
**Materials and methods:** A total of 1513 type 2 diabetic patients with DN were enrolled in the RENAAL study, a multinational, double blind, randomized, placebo controlled study, designed to assess the renoprotective effects of the angiotensin-II-receptor antagonist losartan in type 2 diabetic subjects with DN and were studied for a mean of 3.4 years. LVH was diagnosed by estimating the Cornell product and Sokolow-Lyon voltage using the electrocardiogram (ECG) taken prior to and before leaving the study. The following endpoints were analyzed: primary composite endpoint [doubling of serum creatinine (DSCR), endstage renal disease (ESRD) or death], the various components of the primary endpoint, i.e. ESRD or DSCR, death alone, ESRD or death, ESRD alone, or DSCR alone as well as any CV event.
**Results:** 187 (12%) subjects had LVH at baseline. Treatment with losartan as compared to placebo resulted in a significantly greater decrease in both the Cornell product (-6.2%, P=0.007) and Sokolow-Lyon voltage (-6.3%, P<0.001). The presence of LVH was shown to be significantly associated with the primary endpoint [hazard ratio (HR) 1.42, 95% confidence limits 1.15-1.75, p <0.001], ESRD or DSCR (HR 1.41), death (HR 1.36), CV morbidity and mortality (HR 1.42) and DSCR (HR 1.38). Losartan treatment of subjects with or without LVH caused a significant decrease in the risk for the primary endpoint, ESRD or DSCR, ESRD or death, ESRD and DSCR. Group comparisons showed that patients with LVH receiving placebo had significantly increased risk for the primary endpoint (HR 1.44), ESRD or DSCR (HR 1.42), death (HR 1.59) and CV events (HR 1.68) as compared to those subjects in the placebo group without LVH. Losartan treatment of those subjects with LVH resulted in a cardiorenal risk (primary endpoint, HR 1.04; ESRD/death HR 0.84; CV events HR 1.07) similar to that seen in placebo patients without LVH (HR 1.0).
**Conclusion:** LVH, as diagnosed on ECG, in type 2 diabetic patients with DN is associated with a significantly increased risk for not only death and CV events but also for progression of kidney disease. Losartan reduced LVH in this population and reduced the increased risk of CV and renal events seen in those subjects with LVH.

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**Long-term renoprotective effect of epalrestat, an aldose reductase inhibitor, in Type 2 diabetic patients with microalbuminuria: a 10 years follow-up study**

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**Background and aims:** An accelerated polyol pathway has been implicated in the pathogenesis and development of diabetic complications including nephropathy. We and other investigators have demonstrated that the polyol pathway potentially linked to diabetes-induced renal cell dysfunction through increase in de novo synthesis of diacylglycerol,protein kinase C activity,enhanced production of TGF-beta1,extracellurar matrix proteins, and prostagrandines. Recently, a report has shown that epalrestat may be benefical in preventing diabetic complications by decreasing plasma carboxymethyl-lysine, one of the advanced glycation end products, in diabetic patients.
We have suggested that 5-year administration of epalrestat, an aldose reductase inhibitor retards the progression of incipient diabetic nephropathy. The aim of the present study was to elucidate the long-term effect of epalreatat on renal function in type 2 diabetic patients with microalbuminuria.
**Methods:** Twenty-two type 2 diabetic patients were selected from the Diabetology Division of our department for this study. The patients were diagnosed as microalbuminuric with a urinary albumin excretion (UAE)>30,<300 mg/g.Cr on at least three separate occasions over a 3-month period at beginning and were allocated to one of two groups: 8 patients (epalrestat group) were treated with epalrestat (150 mg/day) orally for 10 years, and 14 patients matched for age,BMI,duration of diabetes mellitus,blood pressure, HbA1 c, serum creatinine and degree of UAE did not receive epalrestat (control group). Data are presented as the mean ±SEM.
**Results:** In the control group, of UAE computed to a logarithm (log UAE) significantly increased from 1.830 ± 0.072 at baseline to 2.470 ± 0.043 after 10 years (p<0.0001). In the epalrestat group, logUAE remained virtually stable during the observation period (1.969 ± 0.089 at baseline to 1.999 ± 0.117 after 10 years).
None of the patients in the epalrestat group developed to overt proteinuria. On the other hand,renal function expressed as reciprocal serum creatinine (1/Cr) significantly decreased from the base value of 1.81 ± 0.13 to 1.33 ± 0.10 at 10 years in the control group (p<0.005), and also from 1.79 ± 0.20 to 1.524 ± 0.150 (p<0.05) in the epalrestat group. However, reduction rate of 1/Cr in the patients treated with epalrestat (25.1%) during 10 years was significantly (p<0.01) smaller than that in the control group (11.8%). No significant changes were seen in blood pressure,HbA1 c,and total cholesterol in both groups during the observation period.
**Conclusion:** The present study suggested that epalrestat may have long-term renoprotective effect against the progression of diabetic nephropathy in type 2 diabetic patients with microalbminuria.

Baseline clinical characteristics and laboratory data of the subjects

| Characteristics | Control group | Epalrestat group |
|---|---|---|
| Sex(M/F) | 7/7 | 4/4 |
| Age(years) | 63.9 ± 2.5 | 62.9 ± 4.8 |
| BMI(kg/m2) | 22.1 ± 0.8 | 23.0 ± 1.3 |
| Known duration of diabetes(years) | 12.1 ± 1.1 | 14.4 ± 2.7 |
| Retinopathy(normal/simple/ preproliferative/proliferative) | 8/4/2/0 | 3/2/3/0 |
| Systolic blood pressure(mmHg) | 132.1 ± 3.3 | 139.3 ± 2.3 |
| Diastolic blood pressure(mmHg) | 77.8 ± 3.3 | 82.2 ± 1.3 |
| HbA1 c(%) | 7.9 ± 0.4 | 8.2 ± 0.5 |
| Serum creatinine(mg/dl) | 0.60 ± 0.04 | 0.67 ± 0.10 |
| Total cholesterol(mg/dl) | 194 ± 9 | 186 ± 9 |
| Urinary albumin excretion(mg/g.Cr) | 83.3 ± 16.3 | 106.3 ± 15.1 |

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**Aldosterone escape during angiotensin II receptor blockade in diabetic nephropathy is associated with enhanced decline in GFR**

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**Background and aims:** Aldosterone has been suggested to play a role in the progression of renal disease independent of arterial blood pressure and plasma angiotensin II levels. Plasma aldosterone levels may be reduced, remain unchanged or even increase (the aldosterone escape phenomenon) during blockade of the renin-angiotensin-aldosterone system (RAAS). The aim of our study was to evaluate the influence of plasma aldosterone levels on progression of diabetic nephropathy during long-term RAAS-blockade.
**Materials and methods:** After a 4-week washout period, 63 hypertensive type 1 diabetic patients with diabetic nephropathy were treated with losartan 100 mg daily, with a mean follow-up of 36 months. Plasma aldosterone, glomerular filtration rate (GFR), albuminuria, and 24-h blood pressure were determined at baseline and regularly during the study.
**Results:** Plasma aldosterone was (geometric mean (95% CI)) 77 (62 to 95) at baseline, compared to 72 (61 to 84) at 2 months and 67 (56 to 80) pg/ml at the end of the study (NS). Patients were separated according to increasing or decreasing levels of plasma aldosterone during long-term ARB treatment. In 26 patients aldosterone levels increased from 57 (43 to 76) at 2 months to 102 (78 to 134) pg/ml at the end of the study (p<0.01). In 37

patients levels decreased from 83 (69 to 102) at 2 months to 49 (40 to 60) pg/ml at the end of the study ($p<0.01$). No difference in plasma aldosterone at baseline was found between the two groups. In patients with increasing aldosterone, rate of decline in GFR was (median (range)) 5.0 (0.4 to 15.9) as compared to 2.4 (-1.6 to 11.0) ml/min/year in patients with declining aldosterone ($p<0.05$). The change in plasma aldosterone correlated with the rate of decline in GFR ($R^2=0.19$, $p<0.01$), corresponding to a decline in GFR of 1.4 ml/min/year for every 2-fold increase in plasma aldosterone. This finding was independent of other progression promoters such as $HbA_{1c}$, albuminuria and blood pressure. No correlations were found between aldosterone levels at baseline or 2 months and rate of decline in GFR. Changes in aldosterone levels were not related to albuminuria or changes in albuminuria.
**Conclusion:** Our data suggest that increasing levels of plasma aldosterone during long-term RAAS blockade (escape phenomenon) is associated with an enhanced decline in GFR in type 1 diabetic patients with diabetic nephropathy.

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**Clinical investigation of pyridoxamine in Type 1 and Type 2 diabetic patients with overt diabetic nephropathy (PYR-205/207)**

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**Background and aims:** Pyridoxamine (Pyridorin™, PYR), delays the progression of diabetic kidney disease in animal models and is a potential therapy for diabetic nephropathy in humans. We present results from a phase 2 clinical investigation of PYR's safety and efficacy in patients with overt nephropathy associated with type 1 and type 2 diabetes.
**Materials and methods:** The PYR-205/207 studies were randomized, double-blind, placebo-controlled, multicenter trials which examined the safety and efficacy of up to 250 mg bid PYR in patients with Type 1 and Type 2 DM and overt nephropathy. The PYR-205/207 studies were identical in design except for entry criteria for serum creatinine (≤ 177 µmol/L in PYR-205 and > 177 but ≤ 310 µmol/L in PYR-207) and were prospectively designed to be analyzed together. Eighty-four patients (17 Type 1, 67 Type 2) at 18 sites were randomized to receive either PYR or placebo in addition to standard of care therapy for six months. PYR dose was escalated from 50 mg bid to 250 mg bid over a 4-week period.
**Results:** Groups were well matched at baseline for age, race, gender, blood pressure, HbA1C, urinary albumin excretion, and ACEI/ARB use. Mean serum creatinine was 169.0 µmol/L in the PYR group and 172.6 µmol/L in the placebo group (NS). No significant differences in treatment-related adverse events (35.1% PYR, 44.4% placebo), treatment-related serious adverse events (1.8% PYR, 0% placebo) or study discontinuation due to adverse events (8.8% PYR, 7.4% placebo) occurred during the studies. The mean rate of rise in serum creatinine was 13.6 µmol/L/yr in the PYR group and 56.6 µmol/L/yr in placebo ($p=0.045$, ANOVA). In Type 2 patients taking either an ACE-I or ARB with a baseline serum creatinine ≥ 115 µmol/L (predefined subgroup), the rate of rise in serum creatinine was 12.7 µmol/L/yr in the PYR group and 105.3 µmol/L/yr in the placebo group ($p<0.0001$, ANOVA). In the same population, the rate of decrease in creatinine clearance was -6.4 mL/min/year in the PYR group and -12.3 mL/min/year in the placebo group ($p=0.017$, ANOVA). A marked treatment benefit was also observed in the mean level of urinary TGFβ1.
**Conclusion:** PYR treatment was safe and well tolerated and inhibited the progression of renal disease in diabetic patients with overt nephropathy. The safety and efficacy results from PYR-205/207 corroborate the results observed in an earlier phase 2 investigation (PYR-206). The beneficial effects of PYR on renal disease progression merit further evaluation in phase 3 trials.
*Sponsor: BioStratum, Inc*

# OP 41

# Novel markers of autoimmunity

## 233

**Mature high affinity immune responses to insulin precipitate the autoimmune cascade leading to Type 1 diabetes**

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**Background and aims:** Autoantibodies to insulin (IAA) are the first immunological markers to appear in young children at risk for Type 1 diabetes (T1D). Many, but not all IAA positive children develop multiple islet autoantibodies, which are associated with high T1D risk. This study was to determine whether IAA affinity matures during preclinical diabetes and whether it predicts progression to multiple islet autoantibodies and T1D.
**Materials and methods:** IAA affinity was measured in the first IAA positive sample and subsequent samples from 56 children followed from birth in the BABYDIAB study cohort. These included 16 children who were first positive at age 9 months, 26 at age 2 years, and 14 at age 5 or 8 years. Of these, 38 developed multiple antibodies and 20 T1D. Affinity was measured in a competitive radiobinding assay using human $Tyr^{14}A$ [$^{125}$I]insulin (10 µCi/ml; 0.143 nM) and increasing quantities of unlabelled human insulin ($2.6 \times 10^{-14}$ to $17.2 \times 10^{-5}$ moles).
**Results:** IAA affinity in the first IAA positive sample ranged from $<10^6$ to $>10^{10}$ L/mol. Affinity in the first positive sample was not associated with IAA titer, but was associated with the age of IAA appearance (median affinity: $8.4 \times 10^9$ L/mol at 9 months, and $5.4 \times 10^9$ L/mol at 2 years; vs. $6.3 \times 10^8$ L/mol at 5 or 8 years; $P=0.005$) and with whether children developed multiple islet autoantibodies (median, $7.4 \times 10^9$ L/mol; IQR, $4.6 \times 10^9$ - $1.0 \times 10^{10}$ L/mol) or remained single IAA positive (median, $1.2 \times 10^8$ L/mol; IQR, $3.6 \times 10^7$–$1.3 \times 10^9$ L/mol; $P<0.0001$). IAA affinity did not change upon follow-up except in one child who had transient IAA ($2.4 \times 10^8$ L/mol) at age 2 years and (re)-developed IAA ($1.6 \times 10^{10}$ L/mol) and GAD antibodies at age 5 years. IAA affinity above $10^9$ L/mol (high affinity) were found in 36 of 38 children who developed multiple autoantibodies including all 20 children who developed T1D, and in 5 of 18 children who had not developed multiple antibodies ($P<0.0001$). Risks for progression to multiple islet autoantibodies (in children with single IAA in the first sample; n=33) and diabetes (in all children) within 3 years of follow-up were 66% and 34% respectively in children with high affinity IAA compared to 0% (multiple antibodies; $P=0.004$) and 0% (diabetes; $P=0.04$) in children with IAA affinity below $10^9$ L/mol.
**Conclusion:** These data indicate that IAA affinity in children with diabetes-relevant IAA is high already from a very young age and does not change, and that the affinity of IAA can identify IAA positive children with high diabetes risk.
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**Residues 626-630 of the IA-2 protein constitute the epitope of mAb 76F4B, a monoclonal antibody representative of Type 1 diabetes-associated autoantibodies**

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**Background and aims:** In type 1 diabetes, IA-2 represents a major actor of humoral immunity since autoantibodies to IA-2 appear in most patients and sometimes several years before clinical onset. The aim of this study was to identify and characterize the IA-2 epitopes which are the most frequently recognized by autoantibodies in the serum of diabetic patients.
**Materials and methods:** We used the recombinant intracytoplasmic domain of IA-2 produced in a baculovirus/insect cell system, three murine anti-IA-2 mAbs (76F4B, A9 and 76B8F) and a panel of 56 sera from diabetic patients. We first investigated by ELISA the inhibition of the binding of the mAbs to recombinant IA-2 by type 1 diabetes and control sera. Continuous epitopes recognized by the three mAbs were mapped 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). The kinetic parameters of the binding of the monoclonal antibodies to recombinant IA-2 was studied by Biacore technology.

**Results:** The serological study demonstrated that 50% (28/56) of type 1 diabetic sera having anti-IA-2 autoantibodies were able to inhibit the binding of 76F4B to IA-2. Therefore, the epitope recognized by mAb 76F4B is representative, in part, of the epitopes recognized by disease-associated autoantibodies. Remarkably, 64% of sera from patients with a disease onset older than 1 year were inhibitory but only 7% of new onset sera. In contrast to mAb 76F4B, the inhibition of the binding of IA-2 to mAbs A9 and 76B8F by the diabetic sera was not significant. Peptide scanning experiments (Spot technique) demonstrated that the epitope recognized by mAb 76F4B is $^{626}$FEYQD$^{630}$ (intracytoplasmic domain). mAb A9 mapped residues $^{716}$LCAYQAE$^{722}$ (intracytoplasmic domain) and mAb 76B8F recognized $^{478}$QKPLS$^{482}$ (intragranular domain). An alanine scanning experiment indicated that residues $^{626}$F, $^{628}$Y and $^{629}$Q are crucial for the binding of 76F4B mAb, whereas $^{719}$Y and $^{720}$Q are key residues for A9 mAb binding. Biacore experiments showed that the affinity of both monoclonal antibodies was quite similar with a $K_D$ of 9.17 x $10^{-8}$M and 9.53 x $10^{-8}$M for mAb 76F4B and A9, respectively.

**Conclusion:** Among the three monoclonal antibodies that we studied, mAb 76F4B was found to be the most representative of type 1 diabetic patients' autoantibodies. Its epitope is localized in the intracellular domain of IA-2 and precisely in the juxtamembrane domain (between the transmembrane region and the PTP domain). A greater percentage of autoantibodies binding to this region was detected in the serum of late onset diabetic patients than in new onset diabetic patients, and this observation might be useful for diagnostic purposes.

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**Soluble B7-1, a newly discovered B7 protein, is elevated in serum of Type 1 diabetic patients**

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**Background and aims:** Type 1 diabetes (T1D) is a polygenic autoimmune disease whose penetrance is controlled by environmental factors. Of the 20+ established and proposed genomic coordinates associated with the risk to develop T1D, few are fine-mapped to candidate genes, none is fully understood. Possibly abnormal B7/CD28 costimulation has been suspected in the induction and progression of autoimmune diseases such as T1D as this costimulation pathway is critical for T-cell activation and proliferation. Recent studies suggested that naturally occurring soluble forms of both B7-1 and B7-2 may exist, but transcrips for the human molecules have not been discovered.

**Results:** We report the discovery of a novel, soluble B7.1 protein. This isoform lacks the two exons coding the transmembrane and cytoplasmic domains, and uses an intron sequence for translation of a new C terminal with a natural stop codon and splicing of the natural B7.1 poly A region. Expression of this soluble B7-1 was detected by mass spectroscopy of sera, using Surfaced Enhanced Laser Desorption/Ionization (SELDI) technology (Ciphergen Biosystem, Fremont, California), and a specific array chip loaded with anti-human B7-1 antibody. With this approach, we observed significantly elevated levels of the new B7-1 protein in serum of 8 patients with new onset T1D, and 10 subjects with autoantibodies and probably pre-T1D. Sera from 17 matched healthy controls showed very low or undetectable amounts of soluble B7-1. Soluble versions of B7.1 (or of its ligands) have been previously generated by molecular engineering and were shown to have major effects on immune responsiveness. Recombinant soluble B7-1 will help us to study the binding affinity of this protein towards the CD28 and CTLA-4.

**Conclusion:** Based on preliminary data, we believe that our discovery has potential to provide a new marker of prediabetic autoimmunity, and soluble B7-1 may directly play a role in immune abnormalities associated with progressive prediabetes.

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**Analysis of eluted peptides from Type 1 diabetes-susceptible HLA class II molecule identified novel islet protein, heparin/heparan sulfate-interacting protein**

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**Background and aims:** Type 1 diabetes is a T cell-mediated autoimmune disease and its susceptibility is strongly conferred by MHC molecules. Activation of autoreactive T cells is thought to be performed by presentation of islet-derived peptides by type 1 diabetes-susceptible HLA molecules. To date, search for islet-derived antigenic peptides presented by type 1 diabetes-susceptible MHC molecules was based on the information of affinity between peptides and MHC molecules. Analysis of naturally processed and presented peptides by MHC molecules was limited to that about established autoantigen recognized by autoantibodies. In this study, we tried to identify islet-derived peptides presented by type 1 diabetes-susceptible HLA molecules from broad spectrum of proteins existed in islet cells.

**Materials and methods:** From a 22-year-old type 1 diabetic woman, B cell line was established by Epstein-Barr virus transformation. B cells were cultured up to $10^{10}$ cells using roller bottles in 2 sets. One set of culture was pulsed with the debris of human fetal islet cell line (1B2C6) and the other set was cultured without pulse. Cells were treated with lysis buffer containing 1% Nonidet P -40, and HLA-DR and DQ molecules were obtained by affinity chromatography. These HLA molecules were treated with 2.5 M acetic acid and low-molecular-weight fractions were isolated by Centicon-10. Then, these fractions were applied to reverse-phase high performance liquid chromatography (HPLC). The HPLC peaks that specifically appeared in pulsed sample were sequenced by Edman degradation methods. Furthermore, pancreatic sections were immunostained using antisera against this peptide, which was obtained by immunization of a guinea pig. HLA-DR and -DQ typing was performed by PCR-RFLP methods.

**Results:** This patient had HLA-DR4 (DRB1*0405/*0407), DQA1*03-DQB1*0302/ DQA1*03-DQB1*0302. Sixty and 990 μg of DR molecules were obtained from non-pulsed and pulsed culture, respectively. Forty-seven and 133 μg of DQ molecules were also obtained from non-pulsed and pulsed culture, respectively. HPLC analysis of the peptides derived from DR molecules revealed 3 HPLC peaks that specifically appeared in pulsed samples. No pulse-specific peak was obtained in HPLC analysis of the peptides derived from DQ molecules. Sequencing of these 3 peaks identified a peptide that consisted of 14 amino acids of AKSXNHTXXNQXRK (X means undetermined amino acid). Homology search found that this peptide derived from heparin/heparan sulfate-interacting protein (HIP). Immunostainig of pancreatic section using antisera against HIP peptide showed exclusive staining of islet.

**Conclusion:** By pulse of whole islet cells (1B2C6), HIP peptide was identified as a naturally processed and presented antigen by type 1 diabetes-susceptible HLA-DR4 molecules. Specific localization of HIP in the islet was also confirmed.

# OP 42

# Glucagon: mechanisms of action

## 237

**Miniglucagon: an unexpected insulin's partner in the peripheral effects of the hypoglycemic hormone**

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**Background and aims:** In the islets of Langerhans, miniglucagon (MG), present in the secretory granules of the α cells and co-secreted with glucagon, exerts an inhibitory tone on insulin secretion. On the other hand, MG, produced from circulating glucagon in its peripheral target tissues, displays original biological effects, mostly opposite to that of the mother-hormone. In an attempt to evaluate the possible importance of MG in the physiological regulation of glycemia, our aims were 1) to evaluate the possible *in vivo* role of MG on insulinemia and glycemia, 2) to address the question of an effect of MG on the insulin signaling pathway in adipose tissue, susceptible to provide a mechanistic explanation for the *in vivo* observations.
**Materials and methods:** *In vivo* studies were performed after catheter installation in jugular vein of Wistar male rats. Plasma glucose levels were determined using a glucose analyzer and plasma insulin was measured by radioimmunoassay. *In vitro* biological studies were performed using 3T3-L1 cells differentiated into adipocytes. Insulin-sensitive GLUT4 translocation to the cell surface was identified by immunofluorescence microscopy. Using immunoprecipitation (IP), $PI_3$ kinase activity was evaluated by measuring the incorporation of [γ-$^{32}$P]ATP on the phosphatidylinositol substrate using autoradiography. Phosphorylated states of IR, IRS and Akt (Protein-kinase B) were evaluated by western blotting and/or IP, using specific antibodies.
**Results:** After an intravenous glucose challenge (1g/kg) in vigil rats, a 5-min MG perfusion (300 pmoles/kg) decreases insulinemia by about 40% with no change in glycemia. This suggests that MG might help insulin, directly or indirectly, in its effects on peripheral tissues. In view of the importance of the glucose transport in the regulation of glycemia in the fat tissue, we have studied at what level(s) of the insulin signaling cascade MG might operate, using the 3T3-L1 adipocyte model. We observed that 1) both MG (100 pM) and insulin (100 ng/ml) stimulate the $PI_3$ kinase activity; 2) both MG and insulin stimulate Akt, *via* the $PI_3$ kinase pathway (inhibition by 100 nM wortmannin); 3) a 3-min treatment by MG (100 pM) induces translocation to the plasma membrane of the GLUT4 glucose transporter, while glucagon (1 nM) has no effect; 4) a 3-min pre-treatment by MG (100 pM) increases the insulin (3 ng/ml) -induced GLUT4 translocation; 5) the analysis of proteic complexes by immunoprecipitation shows that, in contrast to insulin, MG does not phosphorylate the insulin receptor (IR) or the insulin receptor substrates IRS1 or IRS2, but phosphorylates on tyrosine residues a 50-kDa protein, which forms a multi-proteic complex with IR and IRS2.
**Conclusion:** MG stimulates the translocation of the glucose transporter GLUT4 in 3T3-L1 cells, in a manner that relies on $PI_3$ kinase, but which differs in the upstream pathway from the IR/IRS1 phosphorylation mechanism classicaly used by insulin. The *in vitro* action of miniglucagon on the insulin signaling pathway is a likely explanation for its observed *in vivo* effects. These results demonstrate that, in sharp contrast to its mother-hormone glucagon, MG acts as an insulin's partner, opening up a new avenue of research in pathophysiology of diabetes.
*Supported by: Fondation pour la Recherche Médicale (FRM), Association pour la Recherche sur le Cancer (ARC)*

## 238

**Glucagon receptor knockout mice display increased insulin sensitivity and improved beta cell function**

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**Background and aims:** To study the role of glucagon in regulation of islet function and glucose metabolism, glucagon receptor knockout mice (GCGR-/-) have been generated. These mice display reduced blood glucose, increased glucose tolerance, alpha-cell hyperplasia, hyperglucagonemia and increased GLP-1 levels compared to wild type (WT) mice. Here, further studies have been performed to examine beta cell function and insulin sensitivity in these mice.
**Materials and methods:** Euglycemic (6 mM), hyperinsulinemic (20 mU/kg*min) clamp studies were performed in anesthetized mice fitted with carotid and jugular catheters. Intraveneous glucose tolerance tests (IV-GTT, 1 g/kg) were performed under anesthesia in sub-groups of mice, combined with GLP-1 (10.4 μg/kg). Furthermore, the insulin-stimulating effects of IV administrations of CCK-8 (18 μg/kg), arginine (0.25g/kg) and carbachol (30 μg/kg) were determined. Finally, pancreatic islets were isolated and incubated for 60 min in the presence of glucose, GLP-1, arginine and/or CCK-8 for the study of insulin secretion in vitro.
**Results:** Basal blood glucose was lower in GCGR-/- compared to WT mice (5.3 ± 0.4 vs. 9.4 ± 0.7 mM, $p<0.0001$), whereas the corresponding plasma insulin was not different (32.2 ± 9.9 μU/ml vs. 8.6 ± 1.4 μU/ml, ns). Mean steady state glucose infusion rate during clamp was elevated in GCGR-/- mice (30.3 ± 10.1 vs. 18.8 ± 3.1 mg/kg*min in WT, $p<0.01$). During the IV-GTT both the acute insulin response (AIR=mean suprabasal 1 and 5 min insulin, 2.3 ± 0.3 vs 0.87 ± 0.10 nmol/l in WT; $P<0.001$) and the subsequent 5-20 min glucose elimination (7.6 ± 1.0 vs 3.1 ± 0.3%/min in WT; $P<0.001$) were markedly increased in GCGR-/-. Also, the AIR to glucose+GLP-1 was still augmented in GCGR-/- (11.2 ± 0.4 vs 7.5 ± 0.4 nmol/l in WT; $P=0.015$). In contrast, AIR to IV-CCK (11 ± 37 vs 124 ± 25 pmol/l in WT, $P=0.023$) and IV-arginine (161 ± 72 vs 568 ± 71 pmol/l in WT; $P=0.001$) were both reduced in GCGR-/- while the AIR to carbachol (736 ± 98 vs 851 ± 115 pmol/l in WT, ns) was not affected in GCGR-/-. Nevertheless, insulin secretion from isolated islets was reduced in response to all the above-mentioned insulin secretagogues, in GCGR-/- mice.
**Conclusion:** GCGR-/- mice have increased whole body insulin sensitivity. A reduced insulin response, as observed in vivo to non-glucose stimuli such as arginine and CCK and in vitro to several secretagogues, including glucose, may reflect adaptation to increased insulin sensitivity. The reduced insulin response in cultured islets from GCGR-/- mice suggests that glucagon may have a stimulatory role on the glucose sensitivity of the beta-cell. In vivo, however, an augmented insulin response to IV-GTT was observed in GCGR-/-, which may be caused by the increased circulating GLP-1 as described previously.

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**Glucagon receptor antagonists normalize glucagon-induced down-regulation of insulin action and expression of metabolic genes in rat heptocytes**

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**Background and aims:** In view of the inappropriately elevated glucagon levels characteristic of type 2 diabetes, inhibiting glucagon action has long been considered as a means of improving hyperglycemia by reducing hepatic glucose production. However, glucagon antagonism in these patients would be expected to have further beneficial effects on insulin action in the liver as well as on normalizing hepatic gene expression. Here we have tested this hypothesis by examining the effects of the potent small molecule glucagon receptor antagonists, NNC 25-2504 and NNC 25-2648, in primary rat hepatocytes cultured in the presence of elevated glucagon.
**Results:** Both NNC 25-2504 and -2648 have a high affinity for the human glucagon receptor with $IC_{50}$ values for inhibition of glucagon binding of 3 and 12 nM, respectively. In cultured rat hepatocytes NNC 25-2504 and -2648 inhibited glucagon induced glucose production with $IC_{50}$ values of 120 and 900 nM, respectively for glycogenolysis and 0.8 and 3 μM for gluconeogenesis. In rat hepatocytes cultured for 20 hours in the presence of 0.5 nM glucagon 2 μM NNC 25-2504 or -2648, accumulation of glycogen in response to increasing concentrations of insulin was measured and it was found that both antagonists were able to overcome the glucagon mediated down-regulation of insulin action in these cells. In hepatocytes cultured in the presence of 1 nM insulin and 10 nM glucagon, there was a 3.5 fold increase in PEPCK mRNA as determined by real-time PCR. Incubation with 4 μM NNC 25-2648 for 20 hours prevented this increase (1.25 fold). Similarly, Western blot analysis demonstrated that the PEPCK and glucokinase protein expression up-and down-regulation by glucagon, respectively, could be prevented with 4 μM NNC 25-2648.
**Conclusion:** In conclusion, these results suggest that in addition to inhibiting hepatic glucose production, glucagon receptor antagonists may have further long-term benefits by improving insulin action and normalizing hepatic gene expression in diabetic patients with inappropriately elevated glucagon.
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**Identification and characterisation of a novel peptidic glucagon receptor antagonist**

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**Background and aims:** According to the bihormonal hypothesis, plasma glucose levels are regulated by insulin and glucagon action. In the diabetic situation, paradoxically elevated levels of circulating glucagon contribute to hyperglycemia in addition to lacking insulin or diminished insulin action. Therefore, antagonism of glucagon action at the receptor level has the potential for a new antidiabetic therapy.
**Materials and methods:** The bioactive component was purified from the dried culture broth of *Streptomyces* sp. (DSM 14996). Following MeOH extraction BI-32169 was isolated by a combination of preparative HPLC and chromatography on Sephadex LH-20. After acidic hydrolysis the amino acids were identified by HPLC after AccQTag™ derivatization. The Structure of BI-32169 was elucidated by NMR techniques and mass spectrometry.
Biological test systems were based on a BHK-21 cell line stably transfected with an expression construct for the recombinant human glucacon receptor. Activity was studied in receptor binding assays antagonizing $^{125}$J-glucagon binding to membrane fractions and cellular assays in which compounds antagonized glucagon induced cAMP elevation.
**Results:** A new bicyclic 19-peptide, BI-32169 (1), has been isolated and identified as a potent and selective antagonist of the glucagon receptor. Its structure has been established by amino acid analysis, mass spectrometry and thorough 2D NMR analysis. BI-32169 consists exclusively of protein amino acids and is cyclized from the side chain of $Asp^9$ to the N-terminus of $Gly^1$. One disulfide bond between $Cys^6$ and $Cys^{19}$ forms a bicyclic structure.
BI-32169 and its methyl ester derivative are found to be able to antagonise $^{125}$J-glucagon binding to the human recombinant glucagon receptor and showed also potent inhibitory activity against the human glucagon receptor ($IC_{50}$ 440 nM and 320 nM, respectively) in a functional cell based assay. A recently described glucagon receptor antagonist, cephalochromin inhibits glucagon stimulated cAMP elevation with an $IC_{50}$ of 20 µM. BI-32169 and its methyl ester derivative are full antagonists of glucagon action and exhibit no agonistic activity. The peptides are selective for the glucagon receptor, activity of GLP-1 is not inhibited.
**Conclusion:** BI-32169 and its methyl ester derivative might function as new and potent tools to further elucidate the role of glucagon in the pathogenesis of diabetes mellitus or to identify new glucagon receptor antagonists.

# OP 43

# Glucose monitoring and closed loop systems

## 241

**Efficacy of closed loop control of blood glucose and characterization of delays based on an implantable IV sensor and intraperitoneal insulin pump**

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**Background and aims:** Closed loop insulin delivery has the potential to significantly ameliorate the quality of life for individuals with type 1 diabetes. This study was designed to quantify delays associated with intravenous (IV) glucose sensing and intraperitoneal (IP) insulin delivery and also to evaluate the ability of an implantable physiologic Insulin Delivery (iPID) system to efficiently control blood glucose.
**Materials and methods:** The closed loop algorithm was evaluated in 4 type 1 diabetic subjects (2M 2F, BMI 24.3 ± 2.7 kg/m² (mean±SEM), duration of diabetes 38.5 ± 10.2 y, age 60.5 ± 1.0 years). Subjects already implanted with an IV glucose sensor linked to an implantable pump (both Medtronic MiniMed) were admitted at ~7 AM in the Research Center for 3 days. At ~ 8AM, following a 5-hour period of constant insulin delivery (0.6 ± 0.1 U/h), subjects received a meal (40 gr carbohydrates (CHO)) and a bolus of insulin (4.2 ± 0.7 U) based on the CHO sensitivity factor of each subject. Blood was sampled every five minutes for the first half hour after the bolus and every 10 minutes thereafter for 3 hours. At the end of the 3 hour period (~ 11 AM), Closed Loop Control (CLC) was initiated using a β-cell emulating algorithm (1st and 2nd phase insulin release) and continued for 48 hours. Meals were served at 8 AM (40 gr CHO), 1 PM (80 gr CHO) and 7 PM (80 gr CHO). Blood glucose was assessed every 10 minutes for the first two hours of meals and every 30 minutes otherwise. Algorithm parameters (magnitude and ratio of 1st to 2nd phase) were empirically adjusted during the experiment based on antecedent glycemia. CLC in one subject was stopped after 24 hours due to inadequate sensor performance.
**Results:** IP insulin kinetics were modeled using a biexponential model (exponential delays $\tau_1$ and $\tau_2$) and sensor dynamics were described using a first order kinetic model of transport (TD) and exponential ($\tau$) delay. The models accurately described IP insulin kinetics ($R^2$ = 0.8, $\tau_1$=34.6 ± 5.9 min, $\tau_2$ = 17.4 ± 4.7 min), insulin metabolic clearance rate (2021.3 ± 709.1 ml/min) and sensor dynamics ($R^2$=0.7, $T_D$=4.1 ± 4.0, $\tau$= 9.2 ± 6.7 min; two sensors not identified due to technical problems).
During CLC (Table), the algorithm kept glucose within 80–240 mg/dl for 84.1% of the time. Algorithm retuning did not change the percentage of glucose >240 mg/dl but increased the percentage within the 80–120 mg/dl range during the final 24 hours. Excluding meals, glucose was <240 mg/dl for 98% of the time.
**Conclusion:** We conclude that the proposed models accurately describe the dynamics associated with IV glucose sensing and IP insulin delivery, thus allowing for optimal closed loop insulin delivery compensation strategies. Furthermore, the feasibility of the iPID system to provide automated control of blood glucose has been demostrated. We anticipate that the iPID performance will be further improved after optimization of the algorithm tuning.

Table 1: Percentage of time spent in various glucose ranges (mean±SEM)

| Glucose range [mg/dl] | <80 | 80–120 | 120–240 | >240 |
|---|---|---|---|---|
| 48 hours of CLC (%) | 5.2 ± 2.0 | 22.5 ± 1.8 | 61.6 ± 3.0 | 10.7 ± 3.6 |
| First 24 hours (%) | 4.7 ± 2.5 | 18.3 ± 2.8 | 67.0 ± 0.6 | 10.2 ± 3.6 |
| Second 24 hours (%) | 5.8 ± 2.5 | 26.7 ± 2.3 | 56.4 ± 5.7 | 11.1 ± 5.0 |
| 0–2 hours after meal (%) | 3.8 ± 2.5 | 13.0 ± 0.6 | 63.4 ± 4.5 | 19.8 ± 6.7 |
| Outside meal (%) | 6.7 ± 2.1 | 31.5 ± 3.1 | 60.0 ± 3.3 | 1.8 ± 1.8 |

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## 242

**Closed-loop subcutaneous insulin delivery based on subcutaneous glucose sensing in adults**

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**Background and aims:** Automated closed-loop insulin delivery can potentially optimize glycaemic control and improve the quality of life for individuals with insulin dependent diabetes. Feasibility of an external Physiologic Insulin Delivery (ePID) system, which emulates normal beta-cell secretion (1st and 2nd phase insulin release), is being tested. Algorithm parameters have been set in proportion to individual daily insulin requirements (DIR).
**Materials and methods:** The ePID system is comprised of a subcutaneous (sc) glucose sensor and a sc insulin pump (both Medtronic MiniMed) communicating every minute through a laptop which accommodates the control algorithm. Six patients were studied (age 48 ± 5yrs, diabetes 21 ± 6yrs, DIR 0.56 ± 0.04 U/kg/day) during a three day ambulatory open-loop continuous glucose monitoring period followed by a 27 h automated closed-loop period under supervised hospital conditions with 20 min venous blood sampling for glucose (Beckman analyzer), insulin (ELISA, ALPCO), and FFA (Wako). Three standard meals and an evening snack were offered on the first day of closed-loop and breakfast the next morning. Closed-loop target glucose was set at 120 mg/dl. The sc glucose sensor inserted in the evening was calibrated (one-point) in the morning before the closed-loop start and was recalibrated if needed after ~12 hours.
**Results:** Average glycaemia was elevated at the beginning of closed-loop (178 ± 32 mg/dl) and target glucose was achieved within 4 hours. The following pre- and two-hour postprandial glucose values were determined: 72 ± 10, 189 ± 16 mg/dl for lunch; 109 ± 21, 168 ± 30 mg/dl for dinner; 123 ± 7, 214 ± 13 mg/dl for breakfast, after a stable night not different from target ($p>0.05$). The overall plasma glucose range during closed-loop was 50–324 mg/dl. According to the protocol orange juice had been offered at glucose values below 60 mg/dl in four instances. Self-monitoring blood glucose during the open-loop period ranged from 35–510 mg/dl including 11 occurences below 60 mg/dl. Total daily insulin used during closed-loop exceeded open-loop amounts by 41% (42 ± 15 vs 59 ± 29 U, $p<0.05$). Still, closed-loop insulin delivery was very well correlated with individual DIR values ($r^2 = 0.95$). Sc insulin kinetics were accurately described applying a biexponential model ($r^2 = 0.83$).
**Conclusion:** Our data provide proof of concept that near normal glycaemia can be reached during the day and maintained over night by the ePID system. Overall glucose excursions had been either decreased or matched in comparison to open-loop control. A strong correlation between algorithm parameter settings and DIR has been established. However, based on current results, an optimized algorithm parameter set will be applied for ongoing closed-loop studies. Adapting algorithm parameters, implementing compensation for insulin kinetics, increasing the mobility of the subjects during the study, and extending the closed-loop duration will further improve ePID performance, potentially lower meal related excursions, decrease insulin use and eliminate hypoglycemic occurences.
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## 243

**Extended use of a new glucose sensor with wireless data transmission: the next generation CGMS**

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**Background:** The next generation CGMS (Medtronic MiniMed) simplifies patient involvement by replacing the CGMS cable and monitor with a small, wearable transceiver. The sensor-transceiver collects and stores sensor signals without requiring the user to enter calibration values. Patients wear the device, self-monitor their blood glucose, and then download the CGMS with a blood glucose meter. The CGMS is retrospectively calibrated and generates detailed glucose trend information. The purpose of this study was to evaluate the functionality of the next generation CGMS and to assess sensor performance over one week.
**Methods and results:** Ten children, ages 5 to 17 years, with type 1 diabetes wore two CGMS devices simultaneously for 7 days. Accuracy endpoints were paired sensor-meter glucose values. Eighteen of 20 sensors were available for analysis, providing 100 days of device experience. Median sensor life was 165.2 hours (134.0 to 167.7 hours), or approximately 6.8 days. There was a median of 1.96 hours (0 to 16.4 hours) of gaps in continuous glucose monitoring that did not contribute to data and were attributed to system events, e. g. calibration error, sensor out-of-range, or sensor disconnect.

| Days of Sensor Wear | ≤1 | 1–2 | 2–3 | 3–4 | 4–5 | 5–6 | 6–7 | Overall |
|---|---|---|---|---|---|---|---|---|
| Sensors (N) | 18 | 16 | 16 | 16 | 16 | 14 | 14 | 18 |
| Correlation (r) | 0.95 | 0.92 | 0.92 | 0.85 | 0.91 | 0.92 | 0.93 | 0.92 |
| Mean Absolute Difference (MAD) | 12.0% | 14.2% | 13.9% | 13.8% | 12.7% | 14.7% | 13.9% | 13.5% |

**Conclusions:** The next generation CGMS provides accurate glucose values and trend information without requiring that the user calibrate the device while it is worn. By eliminating the cable and monitor, this device allows normal daily activities, such as bathing, and demonstrates that in active children the life of the glucose sensor can be worn for extended periods without any deterioration in sensor accuracy.

## 244

**Longitudinal evaluation of a standardized spectroscopic measurement algorithm designed for non-invasive blood glucose monitoring**

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**Background and aims:** The development of a non-invasive blood glucose monitor is widely recognized as beneficial for the management of diabetes. Several spectroscopic methods have been demonstrated to measure blood glucose non-invasively; however, many of the studies failed to test the stability of the measurement algorithm on subjects over long periods of time. In addition, these studies often do not test the robustness of the measurement algorithm when used across devices. The research reported here tested the stability and robustness of a measurement algorithm developed for non-invasive blood glucose measurements based on near-infrared spectroscopy.
**Materials and methods:** The present study included 19 insulin requiring subjects monitored longitudinally for periods ranging from 3 months to 20 months with a mean duration of 13 months. There were 12 males and 7 females, with a mean age and duration of diabetes of 46 years and 25 years. All subjects underwent glucose profiling tests using non-invasive near-infrared GTS blood glucose devices (Sensys Medical, Inc. Chandler, AZ). A total of 25 devices were used with 4 different optical configurations. Data pairs were taken every 15 minutes comparing the GTS values to forearm glucose values using a home blood glucose monitor (HMBG) (TheraSense, Alameda, CA). Subjects were blinded from glucose data collected on the GTS and were only presented data with the glucose values from the HMBG.
**Results:** More than 80% of the 6144 paired data points (GTS v HMBG) fell in the A-region of the Clarke Error Grid, with more than 99% falling in the A&B regions of the Error Grid. No points fell in the C&E region of the Error Grid, and <0.6% fell in the D region. The mean absolute percent error across the range was 12.1%, and a CV% of 13.8.
**Conclusion:** Long-term stability of a spectroscopic measurement algorithm has been demonstrated across multiple subjects for up to 20 months. Robustness of the measurement algorithm has been demonstrated over 25 devices and 4 optical configurations. These results confirm that a single standardized algorithm, used on all subjects and multiple devices, can be developed for non-invasive blood glucose monitoring.

# OP 44

# Clinical care and Type 2 diabetes

## 245

**Association between hyperglycemia and outcomes in patients undergoing cardiac surgery**

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**Background and aims:** To investigate the relationship between hyperglycemia and outcomes within 30 days in adults undergoing cardiac surgery.
**Materials and methods:** Retrospective data were reviewed for 409 consecutive patients undergoing cardiac surgery at Mayo Clinic in Rochester, MN, between June 10, 2002 and August 30, 2002.
**Results:** The mean age was 64+-15 years; 66% were male and 23% had known diabetes at the time of surgery. Mean and maximal serum glucose levels were determined across the intra-operative and 48-hour post-operative periods. The outcomes were a composite of death, infections (sternal wound, urinary tract, graft donor site, sepsis), neurologic (stroke, transient ischemic attack, coma, delirium), renal (acute renal failure), cardiac (new-onset atrial fibrillation, heart block requiring pacemaker, cardiac arrest) and pulmonary (prolonged pulmonary ventilation, pneumonia) complications within 30 days of surgery. Mean, maximal and initial serum glucose values were significantly higher in patients who had any of the multisystem outcomes compared to those who did not (Table 1).
Table 1. Comparison of mean, maximal and initial glucose values in subjects who had any event compared to those with no event

Table 1.

| Serum Glucose | No Event (n=230) | Any Event (n=179) | p-val |
|---|---|---|---|
| Mean | 140 ± 33 | 159 ± 45 | < 0.01 |
| Max | 187 ± 90 | 214 ± 74 | < 0.01 |
| Initial | 114 ± 26 | 124 ± 40 | < 0.01 |

The significant mean differences in serum glucose levels between the groups persisted after adjusting for age, sex, diabetes, procedure type and insulin use in a multivariable analysis ($p < 0.01$). The adjusted mean difference in serum glucose between those who had any event compared to those who did not was higher in all 6 sub-categories of events. The relationship between mean glucose and events was linear suggesting that the event rate increases consistently as glucose levels increase (Table 2).

Table 2. Mean glucose level and event rate
Table 2.

| Mean Serum Glucose (mg/dL) | No. of Patients | Event Rate (%) |
|---|---|---|
| < 100 | 15 | 20 |
| 100–119 | 93 | 34 |
| 120–139 | 89 | 37 |
| 140–159 | 84 | 41 |
| 160–179 | 56 | 50 |
| 180–199 | 33 | 58 |
| ≥ 200 | 39 | 74 |
| p-value | | < 0.01 |
| Adj. OR[1] per 20 mg/dL | | 1.31 |
| 95% CI | | (1.12, 1.55) |
| p-value | | < 0.01 |

[1] Adjusted for age, gender, diabetes, procedure type (CABG vs. other), and insulin use.

**Conclusion:** This investigation shows that even a modest degree of hyperglycemia is associated with more adverse outcomes in patients undergoing cardiac surgery.

## 246

**Risk modeling to predict stress echocardiogram results in asymptomatic diabetes patients without history of ischemic heart disease**

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**Background and aims:** Silent ischemia is common in patients with diabetes. Angioplasty and revascularization improves prognosis for a select group of these patients at risk for myocardial infarction due to high grade coronary artery stenosis. Evidence based criteria are needed to identify patients at high risk for myocardial infarction for whom cardiac stress testing would identify clinically significant but silent myocardial ischemia.
The authors postulated that the probability of a myocardial infarction predicted by a sophisticated mathematic model such as the Mellibase Risk Assessment or the UKPDS Risk Engine would correlate with the probability of a positive exercise echocardiogram in asymptomatic diabetic patients without a history of ischemic heart disease.
**Material and methods:** The comprehensive electronic medical record at the Mayo Clinic in Jacksonville is designed to allow Boolean searches for specific patient characteristics. The charts of patients who had diabetes, age from 40 to 70 and stress echocardiograms from 2001 to 2003 were identified. Patients were included only if they had no history of ischemic heart disease. Exclusions included evaluation for organ transplant.
Stress echocardiograms at the Mayo Clinic are interpreted using well defined criteria that have been previously published. The calculation of risk by either the Mellibase Risk Assessment or the UKPDS Risk Engine relies on multiple factors including age, duration of diabetes, smoking status, blood pressure, lipid profile and A1 c among others. The ADA criteria for recommending stress testing were also applied to the data.
**Results:** A two-sample t-test found a statistically significant difference between the estimated 10 year risk of myocardial infarction for those with positive and negative exercise echocardiograms. Both the Mellibase and the UKPDS risk estimates were proportional to the probability of a positive stress echocardiogram (see Table), although the sample size was not sufficient to reach statistical significance.

| 10 Year Risk of MI by Mellibase Calculation | # of Patients in Risk Group | # of Positive Stress Echo-cardiograms | Percent Positive Stress Tests |
|---|---|---|---|
| < 10% | 48 | 6 | 13% |
| 10–20% | 80 | 15 | 19% |
| > 20% | 19 | 6 | 32% |

All cutoffs in estimated risk that reduced the tested population by more than 25% excluded some patients with a positive stress echocardiogram. No single traditional risk factor (systolic BP, total cholesterol, triglycerides, or A1 c) had a higher predictive value than the Mellibase risk assessment at an estimated risk >15%.
**Conclusion:** Risk assessment programs provide objective criteria for identifying patients with higher probability of silent myocardial ischemia that can be detected by stress echocardiograms. The calculated risk of a myocardial infarction over a 10 year period appears to correlate with the current probability of a positive stress echocardiogram. There is no practical lower limit of risk below which there is no probability of a positive stress test.
*Supported by: Roche Diagnostics*

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**Fasting C-peptide level and its clinical importance in immune-mediated and Type 2 diabetes mellitus**

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**Background and aims:** The β-cell destruction in immune-mediated diabetes mellitus and the insufficient β-cell activity in type 2 diabetes mellitus leads to a decrease in the plasma C-peptide level. The immune-mediated diabetes is subdivided into a rapidly (type 1) and a slowly progressive form (LADA). Objective: to compare the fasting C-peptide levels of patients with LADA, type 1 and type 2 diabetes with different disease duration. In newly diagnosed LADA and type 1 diabetic patients the association of the C-peptide level with the islet cell-specific autoantibody pattern and the HbA1 c concentration at the time of the diagnosis were evaluated.

**Materials and methods:** The plasma C peptide level was measured in 76 LADA (40 male, age: 53.0 ± 14.5 years), 154 type 1 diabetic (79 male, age: 39.0 ± 14.8 years) and 226 type 2 diabetic patients (59 male, age: 57.8 ± 13.6 years). In each diabetic group three subgroups were created based on the duration of the disease: duration ≤ 1 year, 1-10 years, and >10 years. In 22 newly diagnosed LADA and 41 type 1 diabetic patiens the fasting C peptide level and HbA1 c concentration was measured and the islet cell-specific autoantibody pattern (autoantibodies to islet-cell cytoplasma, ICA and glutamic acid decarboxylase, GADA) was evaluated. All data are given in mean±SD

**Results:** The fasting C-peptide levels did not show significant difference among the three LADA subgroups: duration ≤ 1 year 1.036 ± 0.87; 1–10 years 1.184 ± 0.93; > 10 years 0.755 ± 0.96 nmol/l (NS). There was a significant decrease of C-peptide in type 1 diabetic patients with disease duration 1-10 years compared to the first year after the diagnosis with no further significant decrease after 10 years: duration ≤ 1 year 0.888 ± 0.87; 1-10 years 0.290 ± 0.46; > 10 years 0.156 ± 0.26 nmol/l (p 10 years 1.544 ± 1.35 nmol/l (p=0.0029). We compared the C-peptide levels of patients from different diagnostic groups with similar disease duration. In the first year there was no difference between the LADA and the type 1 diabetic groups but following this the C-peptide levels were higher in both LADA subgroups compared to the respective type 1 diabetic subgroups (duration ≤ 1 year NS, 1-10 years p 10 years p=0.0087). The C-peptide levels were significantly lower in all LADA and type 1 diabetic subgroups compared with type 2 diabetic subgroups (LADA vs type 2: p<0.0001; p<0.0001; p=0.0064; type 1 vs type 2: all p<0.0001). In newly diagnosed autoantibody positive diabetic patients (n=61) the patients with single-autoantibody positivity (n=26) had higher C-peptide levels compared to those with multiple autoantibody positivity (n=35): p=0.0045. There was no correlation between fasting plasma C-peptide and HbA1 c levels at the time of diagnosis.

**Conclusion:** 1) An early significant decrease of the C-peptide level was observed in the rapidly progressive type 1 diabetes. There was no significant change in the C-peptide level in LADA during the course of observation. 2) The C-peptide level started to decrease in type 2 diabetes after ten years of disease duration. 3) Patients with autoimmune diabetes having single-autoantibody positivity expressed higher C-peptide levels. 4) There was no correlation between fasting plasma C-peptide and HbA1 c levels at the time of diagnosis in immune-mediated diabetes.

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**Is there a HbA1c threshold for symptoms of chronic hyperglycaemia?**

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**Aims of the study:** In the National Disease Management Programme for Diabetes mellitus type 2 in Germany two different therapeutic goals regarding metabolic control were defined: "Prevention of microvascular complications" for patients with substantial life expectancy and "absence of symptoms of hyperglycaemia" for patients with reduced life expectancy. However, it is uncertain, if a level of HbA1 c can be defined which is needed to guarantee the absence of symptoms. We tried to study the question which metabolic control may be required to achieve the treatment goal "absence of symptoms due to hyperglycaemia" in 800 patients with diabetes Type 1 and 2.

**Methods:** 1) In a structured interview 86 diabetologists were asked at which HbA1 c value more than 50% of patients would suffer from symptoms of chronic hyperglycaemia. 2) 330 patients (46% women) with diabetes (30% Type 1) without serious general diseases or pregnancy or operation in the past 3 months were asked about their symptoms in a structured interview. Seventeen symptoms of hyperglycaemia (thirst, frequent urination, tiredness, low-performance, delay of wound healing processes, loss of weight etc.) were summarized to the total hyperglycaemia symptom score (THSS; maximum 68). The answers could be given according to the frequency and intensity in the last 4 to 6 weeks and were scored between never=0 to always=4. Patients with depression were detected by the DS-Depression-Scale (ZERSSEN). Diabetes treatment satisfaction was measured by the questionnaire by C. Bradley.

**Results:** According to the physicians over 50% of patients with HbA1 c over 9.2% (range 5.5-15.0%) have symptoms of chronic hyperglycaemia. After exclusion of patients with chronic heart failure NYHA III/IV (n=54; 16,3%) and/or depression (n=52; 15,6%) 248 patients with diabetes were analysed (HbA1 c 7.99 ± 1.54 range 4.1-14.5%; age 56.9 ± 14.3y; duration since diagnosis 15.0 ± 10.7y; BMI 29.6 ± 5.8 kg/m$^2$). There was a significant correlation between THSS and HbA1 c (R=0.134; p=0.026), age (R=0.240; p<0.0001); duration since diagnosis (R=0.245; p<0.0001); BMI (R=0.139 p=0.026), DTSQ Bradley (R=-0.122; p=0.046).

There were no differences in the THSS neither comparing the patients with the lowest (n=64; THSS 17.7 ± 8.9; HbA1 c 6.35 ± 0.49) to the highest HbA1 c quartil (n=61; THSS 20.6 ± 9.7; HbA1 c 10.10 ± 1.29) p=0.084 nor comparing the lowest decentil (n=23; THSS 16.9 ± 9.6; HbA1 c 5.90 ± 0.48) to the highest decentil (n=22; THSS 20.3 ± 9.4; HbA1 c 11.50 ± 1.20) p=0.24. In the logistic regression analysis "drinking at night" and "urination at night" were the only significantly associated symptoms in the lowest and highest HbA1 c decentil as a dependent variable.

**Conclusions:** The HbA1 c threshold for symptoms of hyperglycaemia is set at a substantially lower level asking physicians than asking patients. Drinking and urinating during the night were the most reliable of the 17 symptoms of chronic hyperglycaemia we evaluated. According to our study, the absence of hyperglycaemic symptoms cannot be generally defined by a level of metabolic control. Therefore, if very old patients are treated aiming at keeping them free of symptoms, the achievement of this goal must be evaluated assessing the individual symptoms.

# OP 45

# Functional genetics in Type 2 diabetes

## 249

**Insulin resistance in humans is associated with gene expression changes in skeletal muscle**

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**Background and aims:** Insulin resistance is commonly observed in patients prior to the development of type 2 diabetes and may predict the development of the disease. We hypothesised that the insulin stimulated glucose-disposal seen in insulin resistant patients could be reflected by changes in the gene expression profile in skeletal muscle. The aim of this study was to identify the genes that were differentially expressed in the skeletal muscle of insulin resistant subjects.
**Materials and methods:** Gene expression analysis was performed on human skeletal muscle biopsies from five insulin resistant subjects (glucose infusion rate 34.6 ± 2.7 µmol/min/kg fat free mass, BMI 21.7 ± 1.5) and five insulin sensitive, control subjects (glucose infusion rate 84.5 ± 7.9 µmol/min/kg fat free mass, BMI 25.1 ± 5.2).
Two-colour microarray experiments were performed using 19K oligonucleotide arrays (Ramaciotti Centre, Australia). The arrays were scanned using an Axon scanner and the resultant image analysed with GenePix Pro software (Axon instruments). The data was normalised using internal spike controls (Universal ScoreCard, Amersham) and analysed using DiCy, a database application designed to find, and cross compare, data sets based on both functionality and behaviour.
Genes were considered altered if they displayed a >1.2-fold change in insulin resistance as compared to the insulin sensitive controls.
**Results:** A set of up-regulated or down-regulated genes was identified for a particular array and then cross-referenced across the 10 arrays to determine patterns of differential expression. Eleven genes were consistently up-regulated in the insulin resistant samples (9 of the 10 arrays) and five genes were consistenly down-regulated in the insulin resistant samples (7 of the 10 arrays). These genes are listed in Table 1.
**Conclusion:** Whilst all the genes have been subjected to intensive follow up study, glycogenin is of particular interest. The protein is required for glycogen synthesis, which is commonly decreased in NIDDM patients and defects in the transcription of this gene may contribute the insulin resistant state.
Overall, microarray analysis from this study suggests that the expression of several genes in human skeletal muscle is altered in the insulin resistant state.

Table 1. Differentially expressed genes in insulin resistance

| Up-regulated genes in insulin resistance | Down-regulated genes in insulin resistance |
|---|---|
| Paired box 4 (PAX4) | Glycogenin |
| Ubiquitin | Colon carcinoma related protein |
| Proprotein convertase subtilisin/kexin type 2 (PCSK2) | FMR1P binding RNA |
| NAD+ ADP-ribosyltransferase 2 | Splicing factor (SPF45) |
| CXC chemokine | cDNA FLJ13843 |
| Alkaline phosphatase | |
| Hexose-6-phosphate dehydrogenase | |
| Troponin T1, skeletal, slow | |
| Cadherin | |
| cDNA FLJ10437 | |
| Splicing factor 3b, subunit 1 | |

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**Late onset obesity in mice deficient of the serotonin transporter**

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**Background and aims:** Brain serotonin (5-hydroxytryptamine; 5HT) is known to exert important influences on food intake. In mice, low levels of 5HT lead to an upregulation of food intake while central injection of 5HT reduces food consumption. We observed that 5HT-transporter knockout (5HTT ko) mice become obese with ageing compared to wildtype (WT) mice. To further explore the phenotype of late onset obese 5HTT ko mice we investigated various metabolic parameters under normal and fasting conditions. Since the regulation and metabolic effect of 5HT is closely linked to that of brain derived neurotrophic factor (BDNF), we also studied whether this phenotype correlates with cerebral BDNF mRNA levels.
**Materials and methods:** Twenty-two mice (11 WT, 11 5HTT ko) aged 21–23 months were examined. Treadmill activity was monitored for one week. Six mice of each group were put on a fasting regime overnight, while the rest of each group had access to food and water ad libitum. Subsequently, blood was taken for determination of glucose, insulin, corticosterone, leptin or adiponectin serum concentrations. BDNF mRNA levels in cortex, pons, thalamus and hypothalamus were investigated using quantitative Real-Time PCR.
**Results:** Mean body weight was 51+/-1 g in 5HTT ko mice and 41+/-5g in WT mice. Treadmill activity was significantly reduced and food uptake higher in 5HTT ko mice. 5HTT ko mice tended to exhibit features of insulin resistance (insulin = fasting: WT 2.83 ± 0.57, 5HTT ko 3.06 ± 0.92 ng/ml, ad libitum: WT 3.68 ± 1.24, 5HTT ko 8.2 ± 3.4 ng/ml, p=0.39; glucose = fasting: WT 145 ± 14, 5HTT ko 169 ± 17 mg/dl, ad libitum: WT 167 ± 18, 5HTT ko 179 ± 12 mg/dl, p=0.19). Leptin concentrations were higher in 5HTT ko mice (fasting: WT 5.91 ± 1.98, 5HTT ko 10.98 ± 1.91 ng/ml, ad libitum: WT 10.19 ± 1,33, 5HTT ko 11.55 ± 2.2 ng/ml, p<0.05). Adiponectin concentrations appeared to be higher in 5HTT ko mice (fasting: WT 17.07 ± 2.75, 5HTT ko 20.47 ± 3.05 ng/ml, ad libitum: WT 22,66 ± 3,75, 5HTT ko 29.14 ± 5.16 ng/ml, p=0.11) and corticosterone levels not different (fasting: WT 8.95 ± 1.52, 5HTT ko 9.98 ± 1.45 µg/dl, ad libitum: WT 5.28 ± 0.75, 5HTT ko 5.72 ± 0.89 µg/dl, p=0.23). BDNF mRNA levels were significantly increased in the cortex of fasting WT and 5HTT ko mice compared to their littermates fed ad libitum (p<0.001). The absolute levels of BDNF mRNA were higher in fasted WT than in fasted 5HTT ko mice (p<0,05). In the thalamus there was only a slight increase in BDNF mRNA levels in fasted WT mice (n.s.), and 5HTT ko mice had no BDNF increase after fasting. The pons showed an inverse regulation: mice of both genotypes fed ad libitum had significantly higher amounts of BDNF mRNA than fasted ones (p<0.05). There was no change in BDNF mRNA levels in either genotype in the hypothalamus.
**Conclusion:** 1. 5HTT ko mice develop a late onset obesity. 2. The obesity of 5HTT ko mice is paralleled by increased serum leptin levels and appears to be associated with insulin resistant hyperglycemia, although serum adiponectin levels tend to be higher in 5HTT ko mice as well. 3. The reduced cortical BDNF up regulation in fasting 5HTT ko mice may be related to the mismatch of activity and food intake and, thus, may contribute to their late onset obesity. 4. Fasting conditions lead to increased BDNF mRNA levels in the cortex of WT mice, but not to changes in the hypothalamus, which is regarded central to the regulation of feeding

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**Functional genomic resolution of insulin resistance and dyslipidemia and the gene-gene, gene-environment and pharmacogenetic interactions involving a segment of rat chromosome 4**

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**Background and aims:** The polydactylous rat strain (PD) and the BN.SHR4 congenic strain display attributes of metabolic syndrome X. We investigated the linkage of 83 metabolic and morphometric phenotypes in 2 segregating populations differing by the presence of a differential segment of chromosome 4 (RNO4ds) and tested its impact on rosiglitazone (RSG) action in rats fed high-fat, high-cholesterol diet (HFD).

**Materials and methods:** Adult male PD, BN (Brown Norway) and BN.SHR4 rats were assigned to 2 groups per strain - the control groups (n= 7, 5 and 10, respectively) were fed HFD for 4 weeks, the experimental groups (n=7, 6 and 8, respectively) received RSG (Avandia, 0.4 mg/100g body weight) during last 2 weeks of HFD. Oral glucose tolerance test (OGTT) was performed; serum and tissue triglyceride (TG) and cholesterol (CH), free fatty acids (FFA) and insulin (INS) levels were measured. Lipogenesis, glycogenesis and glucose oxidation in tissues were assessed *in vitro* by incorporation of $^{14}$C-U glucose into total lipids of adipose tissue, glycogen and $CO_2$ in m. soleus, respectively. The expression profile of >15,000 transcripts was assessed in epididymal adipose tissue using the Affymetrix RAE230A array. We derived two intercross (F2) populations of PDxBN (n=149) and PDxBN.SHR4 (n=94) inbred strains. At the age of 10 months, male F2 rats were subjected to a protocol, during which weight, OGTT, serum levels of TG, FFA and INS were measured at baseline, after feeding high-sucrose diet for 1 week and after administration of dexamethasone in drinking water (0.026 mg/ml) for 3 days while still on sucrose diet.
**Results:** The BN.SHR4 had significantly lowest CH levels both in serum and muscle (factor $STRAIN_{ANOVA}$ $p<0.001$) and only in this strain RSG elicited significant increase in liver CH content (56.80 ± 2.74 vs. 70.88 ± 2.88 µmol/g; p=0.003) and no increase in visceral adiposity. We observed an impact of RSG on tissue glucose utilization only in PD, where RSG increased lipogenesis (21.25 ± 1.52 vs. 27.54 ± 1.82 nmol/glucose/mg protein/2 h; p=0.02) and decreased glucose oxidation in muscle (40.87 ± 5.85 vs. 22.24 ± 3.11 nmol glucose/g/2 h; p=0.02). Specific gene expression patterns in BN.SHR4 compared to BN and PD for both HFD and RSG treatments were apparent namely for the genes within the RNO4ds, *Cd36*, P-glycoprotein *(Mdr-1)* and interleukin *6 (Il-6)*. This region showed significant linkage to triglyceride levels and 5 glucose tolerance-related traits uniquely in the PDxBN.SHR4 population, while no linkage was found in the other. Overall, there were 6 loci on chromosomes 2, 3, 5, 8 and 14 with a LOD score difference between the two F2s greater than 4.0.
**Conclusion:** The importance of a specific genomic region for pathophysiology of insulin resistance and dyslipidemia in differing environmental contexts is reflected at the levels of transcriptome, genome-wide linkage and *in vivo* physiology. The genes *Cd36, Mdr-1* and *Il-6* were identified as putative positional and transcriptional candidates for the observed phenotypes.
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**Diabetes per se and metabolic state influence gene expression in tissue dependent manner of BB/OK rats**
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**Background and aims:** Several epidemic studies have clearly established that long term near normoglycaemia strongly protects against onset and progression of late complication of diabetes. Therefore, insulin treatment plays a crucial role in determining the life quality of affected individuals. Here we studied the effects of exogenous insulin on gene expression levels in well- and poorly-compensated diabetic BB rats in comparison to non-diabetic rats to find out whether diabetes per se and the quality of insulin treatment have an effect on gene expression and whether it is tissue specific.
**Material and methods:** Six age-matched non-diabetic (110 ± 5 days) and 12 diabetic male BB rats (F65) were used which were kept under strict hygienic conditions and were free of major pathogens. Diabetics were treated either with one daily application of 1U insulin to guaranty survival of rats and to generate a poorly-compensated group or with an insulin implant which continuously released insulin for 4 weeks to obtain a well-compensated group. Body weight and blood glucose were measured at diabetes onset (day 0) and thereafter daily up to 4 weeks diabetes of duration. After 4 weeks insulin treatment, animals were killed and expression of *Yy1*, *Pparγ*, *Nfκb*, *Pref-1*, *Tgfb*, *Il-10*, and *Lepr* was measured in thymus, spleen, liver and heart using RT-PCR (ABIPrism7000).
**Results:** There were no significant differences between well- and poorly-compensated rats regarding blood glucose values and body weight at diabetes onset. However, significant differences were found between well- and poorly-compensated diabetic animals in body weight gain 8 days and in blood glucose 2 days after diabetes onset and beginning appropriate insulin treatment. The body weight of well-compensated rats increased during insulin treatment and was comparable to those of non-diabetics, while poorly-compensated rats neither lost nor gained weight. In thymus, non-diabetic and diabetic rats did not differ in genes studied except *Lepr*. Poorly-compensated rats showed significantly increased *Lepr* levels whereas well-compensated rats showed amounts comparable with those observed in non-diabetics. In spleen, *Pref-1* expression was significantly decreased in poorly-compensated rats compared with well-compensated and non-diabetic rats. In contrast, *Il-10* was significantly higher expressed in poorly-compensated rats than in the non-diabetic control group. In liver, significant differences were only found in expression of *Yy1*, *Nfkb* and *Lepr*. Well-compensated rats showed no differences when compared with the control group whereas poorly-compensated rats showed significantly higher expression of *Yy1*, *Nfkb* and *Lepr*. Differences between the two diabetic groups were found for the relative expression of *Lepr* and *Yy1*. In the heart, significant differences were observed in *Pparγ*, *Nfkb, Il-10, Tgfb* and *Lepr*. An increased expression was detectable by 50% for *Pparγ* and by 25% for *Il-10* in diabetic rats. Well-compensated rats differed significantly from non-diabetics in *Nfkb* and *Tgfb* gene expression. Only one gene showed significant difference between both diabetic groups. *Pparγ* was significantly reduced in well-compensated rats.
**Conclusions:** The insulin treatment compensates not only metabolic disturbances but also changes gene expression profile in BB rats in tissue dependent manner.

# OP 46

# Vascular complications: glucose-related pathogenic mechanisms

## 253

**High glucose and mechanical stretch downregulate α3β1 integrin expression and reduce cell adhesion in podocytes *in vitro***

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**Background and aims:** Proteinuria is the hallmark and an early feature of diabetic nephropathy, a marker of glomerular vascular damage and a powerful risk factor for disease progression. Metabolic and haemodynamic perturbations are determinant of altered glomerular barrier permselectivity to protein in diabetic glomerulopathy. The podocytes play an important role in regulating protein filtration in the glomerulus and their detachment from the glomerular basement membrane is believed to be one of the mechanisms of proteinuria. The mechanisms of this event is however unknown. Downregulation of α3β1 integrin, an adhesion molecule which anchors podocytes to the glomerular basement membrane, may be involved. We studied *in vitro* whether high glucose (HG-25 mM) and cyclical mechanical stretch (S-20% cell elongation, 1 cycle/ second) modulated α3β1 integrin expression and resulted in changes in podocyte adhesion to extracellular matrix components.
**Materials and methods:** Conditionally immortalised murine podocytes (gift from Prof. P.Mundel) were cultured on flexible membrane plates coated with Human Extracellular Matrix (HECM - mixture of collagen IV, fibronectin, laminin, heparan sulphate proteoglycans, 2 μg/cm$^2$) and exposed to HG or normal glucose (5.5 mM-NG) for 7, 14, and 21 days, followed by S or control/non-stretch (NS) experiments during the last 48 hours of glucose incubation. α3β1 integrin expression was assessed by Western immunoblotting and flow cytometry, and cell attachment on collagen IV, laminin 10/11 and HECM by a crystal violet adhesion assay.
**Results:** HG and S did not affect α3 integrin protein expression. By day 21, HG reduced β1 integrin protein expression by 15% ($p=0.05$) and S by 28% ($p=0.002$). The combination of HG and S downregulated β1 integrin expression by 31%, 25% and 48% after 7 days ($p=0.033$), 14 days ($p=0.041$) and 21 days ($p<0.0001$) respectively. Flow cytometry confirmed the changes in β1 integrin expression. Podocyte adhesion was impaired after exposure to HG, S and their combination after 7, 14, 21 days ($p<0.05$) on all extracellular matrix tested.
**Conclusion:** The combination of high glucose and mechanical stretch additively downregulated β1 integrin expression in podocytes after 21 days. β1 integrin downregulation was paralleled by a reduced cell adhesion to extracellular matrix substrates. Both metabolic and haemodynamic forces play a significant role in podocyte detachment via downregulation of β1 integrin expression.
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## 254

**Common polymorphisms of the human glyoxalase-1 gene and subjects with ischaemic heart disease and diabetes**

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**Background and aims:** Advanced glycation endproducts (AGEs) accumulate at an advanced rate in diabetes mellitus and induce changes in the vascular endothelium that may contribute to micro- and macro vascular disease. Indeed, AGEs formation not only alters the chemical properties of proteins (including LDL), but also induce cellular signalling, activation of trnascription factors and subsequent gene expression and are found in atherosclerotic plaques and within the aortas of subjects with diabetes. Methylglyoxal (MG), a highly glycating agent formed by the fragmentation of triose phosphates is an important precursor of AGEs and is detoxified by the glyoxalase system incorporating glyoxalase-1 and glyoxalase-2. Elevated levels of MG are found in subjects with diabetes and vascular disease and overexpression of glyoxalase-1 prevents hyperglycaemia induced AGEs formation. Perturbation in the human glyoxalase-1 gene (GLO1) may result in vunerability to vascular complications through alterations in AGEs accumulation.
**Materials and methods:** We screened 4.5 kb of 5'UTR, the exons and the 3'UTR of GLO1 for polymorphisms using DHPLC and sequenced those samples demonstrating biallelic variation. Common polymorphisms were analysed in 3 separate RFLP studies. The first cohort comprised 280 subjects with ischaemic heart disease and 280 matched controls, the second had 197 subjects with ischaemic heart disease and the third had 187 subjects with diabetes.
**Results:** We identified a common coding SNP at position 20203 (Ala 111 Glu) and a common SNP in the Kozak sequence at position -7 (T to C). In the first cohort, there was no difference in genotype distribution between the subjects and controls for the -7 and 20203 SNPs. Mutant allele frequencies for -7 and 20203 were 52% and 53% respectively. In the second cohort, a significant association between SNP 20203 and cholesterol concentration was identified in subjects with IHD (CC=5.1, CA=5.5, AA=5.1, $P=0.05$) and a significant association between SNPs -7 and fasting glucose, and 20203 and fasting glucose in controls (CC=4.9, CT=5.1, TT=5.0, $P=0.02$; CC=5.1, CA=5.2, AA=5.2, $P=0.01$ respectively). The geneotype distribution for the 20203 SNP was CC=41, CA=110 and AA=48 giving an allele frequency of 69%. In the third cohort, a significant association was found between 20203 and log factor VII concentrations ($P=0.048$). The genotype distribution for the 20203 SNP was CC=38, CA=88 and AA=60 giving a mutant allele frequency of 76%. No significant associations were identified between this SNP and retinopathy or nephropathy.
**Conclusion:** GLO1 demonstrates at least 2 common SNPs which are associated with markers of diabetic vascular disease. Alterations in the ability of glyoxalase-1 to detoxify MG may have repercusions in AGEs burden that causes changes in the vascular risk profile in subjects with diabtes.
*Supported by: Medical Research Council*

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**Inhibitors of advanced glycation end product accumulation ameliorate renal injury in the diabetic apoE knockout mouse**

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**Background:** It has been previously suggested that advanced glycation end products (AGEs) play a pivotal role in experimental diabetic nephropathy. The present study was aimed to investigate the role of AGEs in the development of renal disease in a model of combined hyperlipidemia and diabetes, the diabetic apoE knockout (KO) mouse.
**Methods:** ApoE KO mice were rendered diabetic (streptozotocin 55 mg/kg injections for 5 days) at the age of 6 weeks. Animals were then randomised to be treated with aminoguanidine (AG, 1 g/l in water) or the putative cross link breaker Alteon 711 (ALT-711, 20 mg/kg BW) or left untreated and followed for 20 weeks. At week 20, animals were put into metabolic cages for 24 hours for assessment of albuminuria. Blood was collected for measurement of glucose, HbA1 c, lipids and serum AGEs. Renal injury was assessed by glomerulosclerosis index (GSI) and tubulointerstitial injury (TI). AGEs, the receptor RAGE, a- smooth muscle actin (SMA) staining, macrophage infiltration (F4/80) were assessed by immunohistochemistry. Collagens I and IV, transforming growth factor beta-1 (TGFbeta-1) and connective tissue growth factor (CTGF) were assessed by RT-PCR and immunohistochemistry.
**Results:** Diabetic apoE KO mice developed more severe renal injury than non-diabetic apoE KO mice as assessed by albuminuria, glomerulosclerosis index and tubulointerstitial injury (Table 1). This was in association with increased renal AGE deposition and RAGE gene and protein expression as well as increases in macrophage infiltration, alpha-SMA positive cells, renal collagen accumulation and increases in expression of the profibrotic growth factors TGF-beta 1 and CTGF. Treatment with AG and ALT 711 were similarly effective in reducing GSI and TI as well as albuminuria with no effects on glycemic control, lipids or blood pressure. These renoprotective effects were associated with a decrease in serum and kidney AGE accumulation, reduced infiltration of macrophages and alpha-SMA positive cells, attenuation of diabetes-increased collagen deposition in the kidney and reduced expression of RAGE as well as profibrotic growth factors such as TGF beta-1 and CTGF.
**Conclusion:** AGEs and the receptor RAGE play an important role in mediating renal injury in the diabetic apoE KO mouse, a model combining hyperlipidemia and diabetes. Two disparate approaches to attenuate renal AGE accumulation were renoprotective in this model. * $p< 0.01$ vs apoE. # $p< 0.01$ vs diab apoE

Table 1

| | ApoE KO | Diabetic ApoE KO | Diabetic + AG | Diabetic + ALT 711 |
|---|---|---|---|---|
| N | 20 | 20 | 20 | 13 |
| Albuminuria mg/day | 6 ± 2 | 90 ± 6* | 60 ± 19*# | 56 ± 7*# |
| GSI (score) | 1.1 ± 0.2 | 3.1 ± 0.2* | 1.9 ± 0.4*# | 1.9 ± 0.4*# |
| TI (score) | 0.91 ± 0.08 | 2.08 ± 0.07* | 1.55 ± 0.08*# | 1.56 ± 0.13*# |
| AGE kid % | 2.8 ± 0.3 | 7.6 ± 1.2* | 4.3 ± 1.8*# | 4.5 ± 1.0*# |
| RAGE kid % | 1.8 ± 0.4 | 2.1 ± 0.5* | 0.6 ± 0.2*# | 0.9 ± 0.9*# |
| CTGF RT-PCR | 1 ± 0.2 | 2.9 ± 0.7* | 1.3 ± 0.3# | 0.9 ± 0.3# |
| TGFb-1 glom | 0.4 ± 0.1 | 4.1 ± 0.3* | 0.3 ± 0.1# | 1.0 ± 0.1# |
| TGFb-1 tub | 1.1 ± 0.2 | 2.5 ± 0.3* | 0.8 ± 0.1# | 1.0 ± 0.1# |

## 256

**The relation of diabetes-associated mono-ADP-ribosylation to impaired brain serotonin transmission: effect of nicotinamide**

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**Background and aims:** Numerous experimental arguments appeared suggesting that the abnormal endogenous ADP-ribosylation of proteins might play a role in the development of diabetic neuropathy and its regulation may represent a novel pharmacological approach to the treatment of diabetic complications. However, a link between changes in extranuclear protein mono-ADP-ribosylation and diabetes-related brain dysfunction still need to be verified. We have thus analyzed the impact of experimental diabetes at different stages of its duration on mono-ADP-ribosylation of proteins as well as its relevance to impairment of neurotransmission. In addition, the effect of nicotinamide (NAm) was examined.
**Materials and methods:** All studies were carried out after 4 (short-term) and 10 (long-term) weeks of diabetes (streptozotocin, 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 was assessed in brain synaptosomes. Basal and cholera toxin (CT, 0.3-1.5 μg/ml) - induced mono-ADP-ribosylation was determined by $^{14}$C-ADP-ribose incorporation to synaptosomal proteins.
**Results:** Spontaneous release of serotonin was significantly increased in short-term diabetic rats, whereas it was decreased in long-term diabetes (by 40.7 ± 5.2% and 24.3 ± 4.1% respectively, $p<0.05$). The level of constitutively mono-ADP-ribosylated proteins in short-term diabetes was 34 ± 5.7% elevated vs control, $p<0.05$. In contrast, chronically diabetic animals elicited a reversal of protein modification to values 12 ± 2.8% lower as compared to appropriate control. In group of animals with more acute diabetes CT-induced mono-ADP-ribosylation resulted in 34.3 ± 5.7% enhancement over basal level and it was accompanied by the ability of toxin to further stimulate serotonin release in a dose-related manner but to a less extent vs that of control, $p<0.05$. Under the same conditions these parameters remained unaffected in short-term diabetic group. Possible involvement of the cAMP in mediating diabetes-associated mono-ADP-ribosylation and serotonin transmission derangements was also found using various cAMP agonists. Phosphodiesterase inhibitor (theophylline, 1 mmol/l), cAMP analogs (dibutyryl-cAMP, 8-bromo-cAMP, 0.5 mmol/l), activator of adenylate cyclase (forskolin, 0.01 mmol/l) increased serotonin release in short-term diabetes but exerted significantly less profound stimulatory effect in chronic disease resembling effects of CT, which is known to catalize mono-ADP-ribosylation of stimulatory G protein alpha subunits, $p<0.05$. Exposure of synaptosomes to cAMP/phospholipid-dependent protein kinase inhibitor H7 (0.01 mmol/l) counteracted CT-related effects on release only in group of short-term diabetes suggesting that impaired serotonin release is, at least, dependent on G-protein-mediated phosphorylation events. NAm treatment after 4 weeks of diabetes virtually normalized both mono-ADP-ribosylation and serotonin release as well as synaptosomal response to all stimuli used, $p<0.05$. Long-term diabetes treatment with NAm also antagonized the inhibitory effect of diabetes on the variables studied but only partially.
**Conclusion:** The findings extend the notion that alterations in mono-ADP-ribosylation may be involved as a possible mechanism responsible for the impaired brain function in diabetes and NAm may efficiently protect against ADP-ribosylation-mediated neuronal injury.

# OP 47

# Experimental immunology

## 257

**Preproinsulin is an important, yet not an essential autoantigen in the pathogenesis of Type 1 diabetes in the NOD mouse**

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**Background and aims:** Although experimental autoimmune diseases can be initiated by immunization with a single antigenic determinant, it is still a matter of debate in how far such a scenario is representative of the natural etiology of spontaneous autoimmune diseases, i.e., whether one distinct self-antigen is obligatorily involved in the initiation and, perhaps, even the perpetuation of autoimmune tissue destruction. We have recently shown that glutamic acid decarboxylase (GAD) does not represent an essential autoantigen in the pathogenesis of type I diabetes (DM) in the NOD mouse.
**Materials and methods:** We now investigate the role of an adaptive immune response against preproinsulin (PPIns) in NOD mice transgenically expressing PPIns2 under the control of a hybrid invariant chain/MHC II promotor.
**Results:** We define for the first time the immune response and cross-reactivity to PPIns1/2 epitopes by in-vitro amplified recall responses against overlapping peptides of both proteins. We demonstrate that PPIns2-transgenic mice are tolerant to all epitopes of PPIns1/2 while immune responses to unrelated antigens are unperturbed. PPIns2-tg. mice display normal islet function, islet architecture and glucose metabolism. They display a less severe insulitis at 12 weeks of age and exhibit a delay in development of DM with a much lower overall incidence of DM (18% in tg. mice vs. 88% in controls). Either hematopoetic cells or radioresistant thymic epithelial cells are sufficient to mediate tolerance. We cannot find any signs of an induction of a dominant tolerance by coculture assays, adoptive transfers and in thymic chimeras.
**Conclusion:** This is the first time that tolerance against PPIns has been achieved and can be linked to the prevention of DM in the majority of PPIns2-tg. animals. We therefore conclude that PPIns - in contrast to GAD - is a central autoantigen in the immunopathogenesis of DM in the NOD mouse; however it does not fulfill the criteria of being an essential autoantigen. By disproving that two major autoantigens GAD and PPIns are essential autoantigens, we question the existence of such autoantigens in the pathogenesis of spontaneous autoimmune diseases. However, the finding that recessive tolerance for a single antigen can prevent disease development in spontaneous autoimmune disease in the majority of animals, provides big therapeutic potential.
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## 258

**Cytokine pattern and iNOS expression in different infiltration stages of islets in the LEW.1AR1/Ztm-*iddm* rat, a new model of Type 1 diabetes**

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**Background and aims:** The LEW.1AR1/Ztm-*iddm* rat is a new animal model of type 1 diabetes mellitus (T1DM) which develops a spontaneous insulin-dependent autoimmune diabetes as a result of apoptotic beta cell death. The islet infiltrate as the morphological correlate of the autoimmune process was mainly composed of macrophages and $CD4^+$- and $CD8^+$-T-lymphocytes. It was the aim of this morphological study to investigate the time course of proinflammatory Th1 and Th2 cytokines and the iNOS induction with respect to the stages of islet infiltration during diabetes development until diabetes manifestation.
**Materials and methods:** Pancreases and spleens as reference organs from 45 - 65 day old animals were analysed by immunohistochemistry and by in situ PCR for the proinflammatory, IL-1 beta, IFN gamma and TNF alpha, the Th2 cytokines, IL-4 and IL-10, and Th1 cytokines, IL-2 and IL-6. The iNOS as well as the caspase 3 mRNA expression were additionally studied with respect to an induction by specific cytokines in islets with a low and high degrees of infiltration.
**Results:** Until day 50 the islets of diabetes prone animals did not reveal any signs of infiltration, a cytokine pattern or iNOS induction. At day 50 the

increase of macrophages in the spleen was the first change which normalized after infiltration of the pancreas. The macrophages in the spleen of diabetic animals, however, showed now a TNF alpha expression on the protein and mRNA levels. At day 55 IL-1 beta and TNF alpha expression as well as IL-4 and IL-10 were localized in the immune cells in islets with an infiltration restricted to the periphery. In parallel the beta cells of these islets revealed an iNOS induction and an increase of caspase 3 mRNA expression in their cytoplasm. Parallel to the increasing infiltration over the whole islet the proinflammatory mRNA expression of IL-1 beta and TNF alpha increased and additionally mRNA expression of IL-2 occurred while the mRNA expression of IL-4 and IL-10 was markedly reduced. In severely infiltrated islets accompanied with a massive beta cell apoptosis only the immune cells showed an iNOS expression. One week after diabetes manifestation no signs of infiltration, cytokine expression or iNOS induction were found any more in the end stage islets without beta cells of severely diabetic animals.
**Conclusion:** The LEW.1AR1/Ztm-*iddm* rat is a new model suitable for the elucidation of the autoimmune mechanisms under in vivo conditions. The analyzed cytokine pattern of immune cells could be correlated to the different infiltration stages of islets. Thus proinflammatory cytokines as IL-1 beta and TNF alpha induced an iNOS and subsequently an increase of caspase 3 mRNA expression in the beta cells which may cause the fulminant beta cell apoptosis leading to T1DM.

## 259

**Overexpression of IL-1 receptor antagonist (IL-1Ra) increases beta cell replication and mass in transplanted islets**
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**Background and aims:** IL-1β could contribute to the dramatic beta cell loss that takes place after islet transplantation. IL-1Ra is a naturally occurring inhibitor of IL-1 action and its overexpression protects pancreatic islets from the deleterious effects of IL-1β on beta cell replication, apoptosis and function. The aim of this study was to determine whether viral gene transfer of the IL-1Ra gene into rat islets *ex vivo* could have a beneficial effect on beta cell replication and mass of transplanted islets.
**Materials and methods:** Lewis rat islets were infected for 2 h with $6.25 \times 10^6$ pfu of Ad-IL-1Ra and streptozotocin-diabetic Lewis rats were transplanted with 500 Ad-IL-1Ra infected islets (Ad-IL-1Ra group) or 500 uninfected islets (control group) under the kidney capsule. Grafts were removed 3 (n = 12), 10 (n = 12) and 28 (n = 12) days after transplantation and beta cell replication, apoptosis and mass were determined.
**Results:** 500 islets are an insufficient mass to restore normoglycemia and therefore, all animals but one (IL-1Ra group) remained hyperglycemic until the end of the study. Beta cell replication (determined by BrdU incorporation) was significantly increased in Ad-IL-1Ra group on days 3 (0.78 ± 0.23%), 10 (1.15 ± 0.16%) and 28 (1.22 ± 0.2%) after islet transplantation compared to beta cell replication in normal pancreas (0.24 ± 0.04%; $p< 0.05$). In contrast, in control group, beta cell replication was not increased on day 3 after transplantation (0.41 ± 0.11%), and although it increased on day 10 (0.89 ± 0.18%; $p< 0.01$) it was reduced again on day 28 (0.59 ± 0.10%) in agreement with previous reports of limited beta cell replication with persistent hyperglycemia. Beta cell apoptosis (determined by TUNEL method) was significantly increased in transplanted islets from both groups compared to pancreas. Although Ad-IL-1Ra group showed lower beta cell apoptotic levels than control group, differences did not reach statistical significance. The initially transplanted β -cell mass (1.34 ± 0.03 mg) was similarly reduced in both control (0.32 ± 0.06 mg) and Ad-IL-1Ra groups (0.45 ± 0.10 mg) ($p< 0.001$) on day 3 after transplantation. In Ad-IL-1Ra islet grafts, beta cell mass increased after 10 (1.04 ± 0.091 mg; $p< 0.010$) and 28 (0.8 ± 0.24 mg) days of transplantation. In contrast, beta cell mass of control group was also increased on day 10 after transplantation (0.69 ± 0.12 mg), but it dropped again on day 28 (0.41 ± 0.05 mg) paralleling the evolution of beta cell replication in this group.
**Conclusion:** Islets overexpressing IL-1Ra showed an increased beta cell replication and a preserved beta cell mass after transplantation, that was maintained even after long-term exposure to hyperglycemia.
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## 260

**DNA vaccine to prevent prediabetic NOD mice from developing diabetes depends upon Th2 responses**
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**Background and aims:** Type 1 diabetes is an autoimmune disease resulting from selective destruction of islet beta cells. One general strategy to suppress ongoing autoimmunity is the induction of Ag-specific regulatory Th cells. Interleukin-4(IL-4) and monocyte chemoattractant protein-1(MCP-1) have been proven to favor Th2 responses. We tested whether DNA vaccination encoding islet autoantigen IA-2 with or without plasmid MCP-1/IL-4 can selectively induce regulatory Th2 cells and prevent NOD mice from developing autoimmune diabetes.
**Materials and methods:** The cDNA of human IA2 was inserted into the vector pEGFP .The expression of recombinant pEGFP-IA2 plasmid were verified in vitro using the method of Western blot and immunofluorescent after transfection in RIN5F cell. Four groups of 4~5 week age or four groups of 10~11 week age NOD mice were injected intramuscularly with 100ug pIA2 plasmid or pIL4/MCP-1 plasmid or both respectively. Control group was injected with pEGFP vector. Glucose level of these groups were detected every 1~2 weeks. RT-PCR and immunofluorescent histology were used to manifest the expression in vivo. Insulitis were evaluated on hematoxylin and heosin-stained pancreatic sections. $CD4^+$, $CD8^+$ T lymphocyte were measured with flow cytometry.
**Results:** Among the 4~5 wks age NOD mice group, 40% mice developed diabetes respectively at 32 weeks when treated with IA2 or IL4/MCP-1 plasmid DNA alone. There are no significant differences when compared with control group (60%). But when combined vaccination with these two plasmids DNA, diabetes incidence decreased to 0% that showed significant differences compared with controls ($p<0.05$). Among the 10–11wks age NOD mice group, 12.5% and 42.9% NOD mice treated with IA2 or IL4/MCP-1 plasmid DNA respectively developed diabetes while there were 12.5% diabetic NOD mice in co-immunization group at 35 weeks. The incidences of diabetes are decreased significantly in IA2 and co-immunization treated mice ($p<0.05$) compared with control group (66.7%).
**Conclusion:** Our results showed that co-vaccination with plasmid DNA of IA2 and IL-4/MCP-1 is more effective in abrogating diabetes than treated with one plasmid alone. This study suggested that prevention of diabetes with autoantigen IA2, can effectively induce immune-tolerance depending upon Th2 responses.

# OP 48
# Brain metabolism

## 261

**$^1$H MRS investigation of brain and liver in patients with Type 1 diabetes mellitus**

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**Background and aims:** Our goal is to investigate peculiarities of cerebral and liver metabolism in patients with type 1 diabetes mellitus on the basis of the analysis $^1$H MRS data.
**Materials and methods:** Two groups of patients are studied by MRI and $^1$H MRS by 1,5T Magnetom Vision /Siemens/. In 1st group 53 diabetics (19-65y.o.) without neurological disorders. The 2nd group consists of 60 healthy volunteers (18-73y.o.). $^1$H MRS in the brain are recorded in both hemispheres in the STEAM sequence: TR/TE=1365/135,20 ms, in the liver with TR/TM/TE=600/10/15 ms.
**Results:** In the $^1$H spectra of the brain in patients with diabetes the following signals are identified: N-acetylaspartic acid (NAA) - 2.04 ppm, creatine (Cr) - 3.04 ppm, choline (Cho)- 3.24 ppm, glucose (Glx)- 3.43-3.8 ppm, myo-inositol (mins)- 3.58 ppm, and lactate (Lac)- 1.33 pmm. In the $^1$H spectra of liver the signals of following metabolites are obtained: Cho-3.22 ppm, lipids-2.0 ppm, glycogen (Glc)- 3.5-4.0 ppm and Lac- 1.33 ppm. For the patients of the 1st group significant decreases in NAA and Cr, and increases in Cho, mins and GIx peak areas are observed.
The signals of GIx and Lac in the liver's spectra in diabetics are detected more frequently compared to spectra of volunteers. These findings reflect the loss of capability of a great portion of liver to regenerate and may be due to decreased oxidative metabolism and/or increased glycolysis leading to lactic acidosis in diabetics.
**Conclusion:** $^1$H MRS is very useful for evaluating the disturbances of metabolism in the brain and liver of patients with long-term diabetes.

## 262

**Brain glucose uptake in human insulin resistance: a positron emission tomography study**

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**Background and aims:** Brain glucose uptake is conventionally considered to be insulin independent. However, recent data from our group showed an increase in brain glucose metabolism at basal concentrations of insulin, compared to absence of insulin. Epidemiological studies show an increased risk of cognitive dysfunction and Alzheimer's disease in insulin resistant individuals. The aim of this study was to compare the ability of basal insulin to stimulate brain glucose uptake and metabolism in insulin sensitive and insulin resistant individuals.
**Materials and methods:** 18-fluorodeoxyglucose positron emission tomography (FDG-PET) was used to measure brain glucose uptake in two groups of healthy male volunteers who had been identified as either insulin sensitive or insulin resistant based on HOMA-IR calculated from fasting insulin and glucose concentrations. The insulin sensitive (IS) group (n=6) had a mean HOMA-IR of 1.3 (range 0.92–1.58), mean fasting insulin 6.3 mU/l (range 5–8.5), mean fasting glucose 4.8 mmol/l (range 4.2-5.8) with average BMI 26.5 (range 23.9-30). The insulin resistant (IR) group (n=7) had a mean HOMA-IR of 6.3 (range 2.94–9.5, p=0.0026), mean fasting insulin 25.1 mU/l (range 11.6–41.8, p=0.005), mean fasting glucose 5.7 mmol/l (range 5.1–6.6, p=0.014) and average BMI 28.4 (range 23.7–35.3, p=0.41). Each subject underwent two PET scans in random order. During each scan endogenous insulin secretion was suppressed using somatostatin infusion at a dose of 0.1 mcg/kg/min. Insulin was replaced during one scan only at 0.3 mU/kg/min. Euglycaemia was maintained with 20% glucose infusion if necessary. After 90 min of somatostatin +/- insulin infusion, a single dose of approximately 150MBq FDG was given followed by dynamic scanning over 90 min.
**Results:** Somatostatin suppressed insulin concentration to 2.8+/-0.29 mU/l in the IS group and 0.9+/-0.42 mU/l in the IR group, p=0.13. With insulin replacement insulin concentration was 26.1+/-1.37 mU/l in the IS group and 22.7+/-1.98 mU/l in the IR group, p=0.10. In the IS group, average global cerebral metabolic rate of glucose was 0.209 mmol/kg/min without insulin and 0.247 mmol/l with insulin replacement, p=0.007, an increase of 18.2%. In contrast, preliminary analysis of data for the IR group showed no significant increase in brain glucose uptake (4.4%, p=0.49) with insulin replacement.
**Conclusion:** Our data support the hypothesis that peripheral insulin resistance extends to the brain. The majority of glucose uptake into the brain occurs via insulin insensitive glucose transporters. However, GLUT-1, the principal glucose transporter in glial cells, is partially insulin sensitive, and the insulin dependent transporter GLUT-4 has recently been identified in neuronal tissue. In insulin sensitive individuals, increases in brain glucose uptake due to insulin are superimposed on a background of insulin-independent uptake. The insulin-dependent brain glucose uptake appears to be diminished in insulin resistant individuals. Brain insulin resistance may be relevant to the described link between the development of cognitive dysfunction and insulin resistance.
*The study was funded by Diabetes UK. Dr Anthony was supported by a Diabetes Wellness and Research Foundation Clinical Fellowship.*

## 263

**Identifying the regional brain activation response to oral glucose ingestion using functional magnetic resonance imaging**

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**Background and aims:** Much remains unknown about the brain's involvement in the control of peripheral metabolism and nutrient sensing in man. Functional magnetic resonance imaging (fMRI) is a neuroimaging technique that allows us to visualise brain regions active in particular functions. The aim of our study was to map out normal regional brain activation in response to oral glucose ingestion across the whole brain.
**Materials and methods:** Six fasted right-handed healthy volunteers (four female, mean age 38 years, range 30–47 years) underwent 2 separate fMRI scans. Subjects randomly consumed either 75g glucose in 275 mls water (G) or 275 mls water only (W), followed by 40 min fMRI scanning (gradient echo EPI, 60 axial slices, 2.5 mm thickness). Subjects received a uniform level of constant visual and auditory stimulation throughout each scan. The two studies were repeated outside the scanner at a later date for measurement of plasma glucose and insulin responses at 2.5 min intervals.
**Results:** Plasma glucose concentration increased in G only. The rise became significant at 7.5 min (p=0.03) and continued for the full duration of the scan. There was an associated rise in insulin concentrations following G but not W. Group brain activation maps showed areas of significant change in regional brain activity in 2 regions: amygdala/hippocampus and brain stem/hypothalamus, in G only (p<0.05). Time series analysis of the data in each region showed the change in activation to be M-shaped, with a predominantly negative deflection. At its maximum the deflection was a 2% change in signal, occurring 6 to 13 min after glucose ingestion. This signal change was contemporaneous with the onset in the rise of plasma glucose. There was no change in either region in response to ingestion of water.
**Conclusion:** The negative deflection in each region indicates reduced neuronal activation in response to glucose ingestion. The reduction in brain activity suggests a regulatory function is occurring, which relies on regional de-activation. The brainstem and hypothalamus are known to be associated with responses to food and hunger, autonomic control and glucose sensing. The response in the amygdala/hippocampal brain region is a novel finding, but the amygdala is sensitive to peptides and transmitters that affect food intake and ingestive behaviour is altered following amygdala lesions. The hippocampus is rich in insulin receptors and glucose transporters. The ability to image activation of central glucose sensors can now be used to examine important disease states, including insulin resistance, obesity and hypoglycaemia responsiveness in diabetes.

## 264

**Involvement of 5'-AMP-activated protein kinase (AMPK) in glucose sensing by hypothalamic neurons**

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**Background and Aims:** Both glucose-excited (GE) and glucose-inhibited (GI) neurons of the basomedial hypothalamus are likely to play important roles in the control of satiety and feeding behaviour. At present, the mechanism(s) by which glucose and other stimuli regulate the activity of either neuronal cell type is unclear. Here, we investigate the role of 5'-AMP-activated protein kinase (AMPK), an enzyme whose activity is strongly increased in other cell types upon nutrient deprivation and whose activation has been recently shown to increase feeding in in vivo animal studies.
**Material and Methods:** Cellular voltage responses in whole cell or perforated patch clamp experiments (current clamp configuration) and changes

in intracellular free $Ca^{2+}$ concentration measured with fluo-3 were studied in cultured hypothalamic neurons from three day-old rats.
**Results:** Patch clamp and intracellular free $Ca^{2+}$ experiments revealed that 15% (4 of 26 cells from four different cultures) or 5% (6 of 121 cells from four different cultures) of neurons respectively were activated by glucose deprivation (0 vs 15 mM). The effects of glucose withdrawal on both intracellular free [$Ca^{2+}$] ([$Ca^{2+}$]$_i$) oscillations and cellular voltage responses were mimicked by the addition of the pharmacological AMPK activator, 5-amino-imidazole-4-carboxamide riboside (AICAR; [$Ca^{2+}$]$_i$ responses: n = 6/6 cells from four different cultures, cellular voltage responses: n=3/3 cells from three different cultures).
**Conclusion:** We conclude that changes in AMPK activity may modulate the activity of hypothalamic GI neurons, and might permit these cells to respond to fluctuations in blood glucose concentration via changes in intracellular [AMP]/[ATP] ratio, rather than free [ATP].
*This work was funded by a Wellcome Trust Prize Studentship and Programme grant No. 067081/2/02/7 to Guy A Rutter.*

# PS 1

# Epidemiology: Type 2 diabetes – prevalence and routine registries

## 265

**Height partly explains the gender differences seen in post load glucose – the Ausdiab Study**
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IGT and diabetes diagnosed by the 2 h plasma glucose (2 hPG) are more common in women than men, while IFG and diabetes diagnosed by the fasting plasma glucose (FPG) are more common in men. This is due to a higher 2 hPG, lower FPG and a larger FPG-2 hPG increment in women. The aim of this analysis was to explore these differences, using a nationally representative sample of 11,247 Australian adults. Results from an oral glucose tolerance test (OGTT) and anthropometric measurements were available on 10,410 non-pregnant participants without previously diagnosed diabetes. IGT was more common in women than men (12.0% vs 9.2%), as was diabetes diagnosed by the 2 hPG alone (2.3% vs 1.9%) while IFG (3.3% vs 8.1%) and diabetes diagnosed by the FPG alone (0.7% vs 1.5%) were less common in women. Mean FPG was higher in men than women (5.6 vs 5.3 mmol/l, $p< 0.001$), while women had a higher mean 2 hPG (6.2 vs 6.0 mmol/l, $p< 0.01$), and FPG-2 hPG increment (1.0 vs 0.5 mmol/l, $p< 0.001$). These differences were unaffected by adjustment for age and obesity. After adjustment for age and height, the gender difference in FPG remained, but the differences in 2 hPG reversed so that the mean for men was higher (6.3 vs 6.0, $p< 0.001$), and the difference in the FPG-2 hPG increment disappeared (0.8 vs 0.7 mmol/l, $p>0.1$). After age adjustment, height was strongly related to 2 hPG, but not to FPG (table) in men ($p<0.001$) and women ($p=0.002$). For any given FPG below 7.0 mmol/l, women had a significantly higher mean 2 hPG than men, but $HbA_{1c}$ showed no gender difference. These results show that the greater 2 hPG and FPG-2 hPG increment observed in women is largely explained by their smaller height, and suggest that the glucose load in the OGTT may need to be adjusted for body size.

Mean fasting and 2 h plasma glucose (mmol/l) according to height quartile

| | Height quartile | | | |
|---|---|---|---|---|
| | Q1 | Q2 | Q3 | Q4 |
| FPG – male | 5.48 | 5.51 | 5.58 | 5.55 |
| FPG – female | 5.30 | 5.24 | 5.24 | 5.18 |
| 2 hPG – male | 6.59 | 6.19 | 6.20 | 5.83 |
| 2 hPG – female | 6.36 | 6.17 | 5.92 | 5.70 |

## 266

**Disability in young age and risk of diabetes mellitus**
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**Background and aims:** Prevalence of both Diabetes and Cardiovascular Diseases show inverse associations with income, education and socioeconomic status in western populations. Disability is less investigated, and our aim was to explore the prevalence of Diabetes among young Disability Pensioners, and to compare the findings at different educational levels.
**Materials and methods:** Our material represents subjects from surveys in 11 Norwegian counties during 1997–1999. Participation rate was 60%. In addition to blood samples and basic clinical recordings, the participants filled in a questionnaire on health, diseases, symptoms, risk factors, lifestyle, education and employment. After excluding persons who reported being unemployed, on sick leave or on rehabilitation, 1.386 subjects were categorized as Disability Pensioners and 53.916 as 'healthy'. The educational levels "higher" and "basic" correspond to whether the subjects started education after completing the nine-year obligatory school or not.
**Results:** Prevalence of self-reported Diabetes was significantly increased among Disability Pensioners compared to healthy (3,3% vs 0,8, $p<0,001$). This was found significant among both men and women. The difference was also consistent in the 2 educational levels. OR for Diabetes among Disable Pensioners was found significantly elevated both among men and

women with basic education, 3,8 and 2,9 respectiviely (tab.). Among the higher educated women, OR was not significantly increased, but among men disability was associated with an OR as high as 15,0 (tab).
**Conclusion:** Young Disability Pensioners show increased risk of Diabetes after adjustments of main risk factors. The increased prevalence of Diabetes may have been present prior to the Disability Pension was given, or may be secondary to the disability, or linked with the exclusion from working life. This finding calls for further investigation, and is a challenge for health care providers and population health authorities.

Odds Ratio for DM in Disability Pensioners, adjusted for age, waist and activity

| Gender | Education | Included (n) | DM (n) | Odds Ratio | CI | Sig. |
|---|---|---|---|---|---|---|
| Men | Basic | 14.121 | 119 | 3,8 | 1,9–7,3 | p<0,001 |
| Men | Higher | 11.153 | 99 | 15,0 | 7,7–29,3 | p<0,001 |
| Women | Basic | 14.537 | 123 | 2,9 | 1,7–4,9 | p<0,001 |
| Women | Higher | 12.708 | 83 | 1,4 | 0,4–4,6 | ns |

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**Obesity: fuelling the diabetes epidemic in Cameroon**
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**Background and aims:** Over the past decade the prevalence of type 2 diabetes in Cameroon has been steadily on the rise. From a prevalence of approximately 1–2% reported in urban and rural areas in 1994, this figure is currently around 5%. Westernisation of the hitherto hunter-gatherer lifestyles that prevailed in this community has been largely implicated in this rising prevalence of type 2 diabetes.
This study therefore set out to look at the possible contribution of this changing lifestyle in the prevalence of overweight and obesity which is associated with insulin resistance and type 2 diabetes.
**Material and methods:** A total of 1257 subjects (375 from a rural area and 882 from an urban area) were recruited into this survey from sites previously used in 1993 to survey the prevalence of type 2 diabetes and its risk factors. Similar sampling methods and field techniques as used in the 1993 survey were replicated in these sites. SPSS 10 for windows was used for statistical analyses. The current WHO recommended body mass index classification was used to define overweight and obesity. Statistical significance was set at $p < 0.05$.
**Results:** The results show a marked increase in the prevalence of overweight and obesity especially in the rural population. About a quarter of the rural population studied was either overweight or obese (table 1) in the current survey, this figure is almost double that of 1993. The shift towards overweight and obesity in the urban population has not been as dramatic as in the rural population, although in both surveys, more than 50% of the urban women and about half of urban men are either overweight or obese (table 2).
In this series we found a relative prevalence of type 2 diabetes of 4.0% in obese compared to 1.1% in normal weight women and 4.2% in obese compared to 0.9% in normal weight men.

**Table 1: Rural population**

| | Women | | | Men | | |
|---|---|---|---|---|---|---|
| Characteristics | Survey 1, 1994 | Survey 2, 2003 | p | Survey 1, 1994 | Survey 2, 2003 | p |
| n | 441 | 207 | | 309 | 168 | |
| Age (years) | 45.8 ± 12.7 | 46.7 ± 14.2 | 0.4 | 45.8 ± 13.2 | 46.3 ± 14.9 | 0.7 |
| BMI (kg/m²) | 22.3 ± 3.3 | 23.5 ± 5.6 | 0.0007 | 22.9 ± 2.7 | 22.9 ± 4.1 | 1.0 |
| Waist (cm) | 82.1 ± 8.7 | 80.7 ± 11.2 | 0.08 | 80.7 ± 8.4 | 79.6 ± 9.6 | 0.2 |
| Hip (cm) | 91.7 ± 8.9 | 95.4 ± 10.9 | 0.0001 | 89.6 ± 7.4 | 90.1 ± 9.8 | 0.5 |
| WHR | 0.90 ± 0.10 | 0.85 ± 0.10 | 0.00001 | 0.90 ± 0.09 | 0.89 ± 0.10 | 0.3 |
| **Classification of obesity** | | | | | | |
| Overweight | 13.0% | 18.6% | 0.004 | 9.3% | 20.4% | 0.001 |
| Obese | 3.2% | 8.0% | 0.002 | 1.7% | 3.1% | 0.003 |
| **Diabetes** | 0.5% | 5.8% * | | 0.9% | 5.2% * | |

* Unadjusted

**Table 2: Urban population**

| | Women | | | Men | | |
|---|---|---|---|---|---|---|
| Characteristics | Survey 1, 1994 | Survey 2, 2003 | p | Survey 1, 1994 | Survey 2, 2003 | p |
| n | 593 | 483 | 460 | 399 | | |
| Age (years) | 37.5 ± 9.2 | 37.8 ± 11.0 | 0.6 | 37.8 ± 9.0 | 35.6 ± 10.2 | 0.0008 |
| BMI (kg/m²) | 27.0 ± 5.0 | 27.1 ± 6.3 | 0.8 | 25.0 ± 3.7 | 24.8 ± 5.1 | 0.5 |
| Waist (cm) | 81.5 ± 9.9 | 89.4 ± 15.1 | 0.00001 | 83.8 ± 9.1 | 86.1 ± 13.5 | 0.003 |
| Hip (cm) | 104.8 ± 10.4 | 104.2 ± 12.7 | 0.4 | 98.5 ± 8.0 | 98.3 ± 12.5 | 0.8 |
| WHR | 0.78 ± 0.06 | 0.86 ± 0.12 | 0.00001 | 0.85 ± 0.04 | 0.88 ± 0.10 | 0.00001 |
| **Classification of obesity** | | | | | | |
| Overweight | 36.1% | 31.7% | 0.03 | 35.7% | 26.3% | 0.006 |
| Obese | 24.7% | 27.5% | 0.05 | 10.2% | 12.8% | 0.05 |
| **Diabetes** | 1.6% | 6.7% * | | 0.8% | | 5.8%* |

* Unadjusted

**Conclusions:** These results show that obesity is a serious public health problem in Cameroon and is a major contributor to the rising prevalence of type 2 diabetes.

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**Applying the WHO STEPS approach in a resource-limited country: the Cameroon Burden of Diabetes Study**
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**Background and aims:** Non-communicable diseases such as diabetes and cardiovascular disease are becoming increasingly important as causes of morbidity and mortality in developing countries. The Cameroon Burden of Diabetes project used the WHO STEP-wise approach to NCD risk factor surveillance to set up a national sentinel surveillance system for Diabetes and its risk factors in Cameroon.
**Materials and methods:** The aim of the project was to provide scientifically robust new knowledge to guide the development of a national policy for the surveillance, prevention and control of diabetes in Cameroon. A cross sectional baseline survey was organised in 4 urban health districts defined as sentinel sites, selected purposively from each of the 4 ecological zones of Cameroon. Data was collected from community household members ≥15 years.
A sample size of 410 subjects per 10-year age group was required (WHO recommends at least 250 subjects per 10-year age group). From 15 to 64 years, the population was divided into five ten-year age groups, then the 65 years and above range constituted an extra group. Thus, $410 \times 6 = 2460$ subjects were required per site. This gave a total sample size of 9840 subjects for the 4 sites. The sampling scheme employed was a multilevel systematic sampling stratified by age group and the sampling unit was households. Data was collected using the WHO STEPS adapted tool. Field activities included census (registration) of eligible individuals, determination of household sampling intervals, administration of household questionnaire and examination of subjects. Data entry and analysis was done in Epi info version 6.0 and with Stata 6.0. Prevalence results were calculated after direct age standardization using the WHO New World Population. The level of significance of p-value was set at 0.05
**Results:** Household respondents: 10,011 subjects were examined with response rate of 95%. The prevalence of diabetes was 5.7% (cp 1.1% in 1994), 5.8% (ci: 5–6.6) for males and 5.5% (ci: 4.80–6.20) for females. The rate was highest in the ≥65-year age group (9.1%, ci: 8.30–17.45). Among the detected cases of diabetes, 80% were newly diagnosed. Seventy four percent of the known cases were on treatment and approximately 27% of the treated cases were controlled. The mean duration of diabetes in known patients was 7 years (5.55–8.70 years). The prevalence of hypertension was 24% (26% in male and 23% in female) and 50% in subjects above 55 years. Sixty six percent of the cases of hypertension went undetected. Of the known cases of hypertension, 46% were treated and 19% of those treated were controlled. Thirty percent of men and 50% of women had a BMI ≥25 kg/m². Eight percent of males and 21% of females had a BMI ≥ 30 kg/m².
Intervening on overweight and obesity could reduce the prevalence of hypertension and diabetes by 24% and 15% respectively in men; and 29% and 13% respectively in women. A majority of the respondent were physically inactive at work place and up to 90% were sedentary at leisure. Alcohol consumption was widespread with 84% of the respondents

reported to have taken at least an alcoholic drink in the last 12 months. The age and sex adjusted prevalence of tobacco consumption was 14.79%.
**Conclusion:** Diabetes is on the rise in the Cameroonian population and may be attributable mainly to increasing obesity. There is thus an urgent need for putting in place a national diabetes control and prevention programme.
*Supported by: WDF*

## 269

**Incidence of gestational diabetes mellitus: results of a validated universal screening**

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**Background and aims:** The incidence of gestational diabetes mellitus (GDM) was found to be 2.5–5.4% during the first three years of a screening in a region of Budapest initiated in 1999 and planned to be universal. The preliminary results suggested a high risk for GDM in the investigated Caucasian population, however, the ascertainment rate was only 47%. Our aim was therefore to try to validate results of the ongoing screening.
**Materials and methods:** Results of one year from 1 May 2002 to 30 April 2003 (screening by 75 g oGTT between the 24$^{th}$–28$^{th}$ weeks of gestation; WHO criteria) were evaluated and validated contacting all women at the ward after delivery. Demographic and risk factor data were collected using questionnaires.
**Results:** During the observed period 1433 women delivered according to the computer system of the hospital, data of 1408 patients were back-collected and examined (mean age: 28.8 ± 4.5 years [±SD]; BMI: 23.1 ± 4.3 kg/m$^2$, Caucasian: 95.6%) with an ascertainment rate of 98%. Among women in our database oGTT was performed in 1288 cases (91.5%). According to the results of this screening 74 patients (5.3%) was found to have GDM while 86.2% had normal carbohydrate metabolism. Only 8.4% of the screened pregnant population had low risk for GDM according to the ADA recommendations (age<25 years, BMI <25 kg/m$^2$, no history of poor obstetric outcome or abnormal carbohydrate metabolism and no known diabetes in first degree relatives). Based on their age, 83.3% did not belong to the low risk group. Among them the frequency of GDM was significantly higher (OR=2.7, 95% CI: 1.1–6.6) compared to those with low age risk. When the BMI was examined similarly, 22.7% of women did not belong to the low risk range (OR=2.4, CI: 1.5–3.8). Diabetes mellitus in first-degree relatives (11.2%, OR=1.7, CI: 0.9–3.1) and history of poor obstetric outcome (42%, OR=0.9, CI: 0.6–1.5) did not play a significant role in the occurrence of GDM. History of known abnormal glucose tolerance (1.8%, OR=1.6, 0.4–7.1) was not a significant risk factor either in this group of women.
**Conclusion:** Based on our validated universal screening with a high ascertainment rate, we conclude that the incidence of GDM in the Hungarian (Caucasian) pregnant population is high (5.3%). The overwhelming majority of women are not at low risk for GDM (primarily due to the increasing age at delivery), therefore, universal screening is absolutely recommended.

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**The diagnostic accuracy of the diabetes register on a prescriptive basis: a comparison between two classification criteria**

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**Background and aims:** To evaluate whether the classification of the diabetic patients obtained by using an administrative source constitutes a valid and reliable classification for the definition of cases of diabetes.
**Materials and methods:** Two classification criteria were used: the clinical criterion (gold standard) using the data present in the Diabetology Unit database and the administrative criterion used for the creation of a diabetes register, defined by analysing the prescriptive dynamics of the antidiabetic drugs (ATC A10 code). Two different thresholds were used: diagnosis of diabetes when there were at least two prescriptions for hypoglycaemic agents in a year, and diagnosis of diabetes when there was at least one prescription in a year. Both classification methods were compared with the Gold Standard. Patients present in both the clinical archive and in the administrative one were included in the study.
**Results:** A total of 3681 patients were present in both archives. In the comparison between the two-prescription classification criterion and the gold standard, of the 3681 patients, 2971 were classified as diabetic with the administrative criterion and 3137 when the diagnosis was based on the gold standard. Sensitivity and specificity were 85.2% and 44.9% respectively, with a diagnostic accuracy of 79.2%. With the one-prescription classification, the number of diabetics identified with the administrative criterion increased to 3106. Sensitivity and specificity were 88.9% and 41.7% respectively, with a diagnostic accuracy of 81.9%. Of the 3137 patients diagnosed as diabetic with the gold standard, 151 (4.8%) were following a diet without taking any type of antidiabetic drug.
**Conclusion:** While the information obtained from the administrative archives presents advantages, it does need appropriate validation. In the diabetes register on a prescriptive basis, the two and one-prescription classification criteria present some limits. In the first, a source of ambiguity is represented by patients with only one prescription of antidiabetic drugs. Although the one-prescription criterion proved more accurate in overall terms, the patients under dietary treatment pass unnoticed with both classification criteria. The study represents a first attempt to validate classification criteria on a administrative basis. The identification of factors that lead to an incorrect classification is of fundamental importance for defining more effective classification criteria and for a correct interpretation of the results.

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**Type 2 diabetes 1996–2003: Results from the Swedish national diabetes register NDR**

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**Background and aims:** Type 2 diabetes (DM2), which is considered a progressive disease, has been reported to increase in incidence in most countries. Recent clinical trials have demonstrated profound effects of risk factor treatment in DM2 and intensive multiple risk factor intervention has been shown to halve the development of diabetic complications. This report addresses if DM2 is increasing in Sweden, treatment patterns and risk factor profile in DM2.
**Material and methods:** NDR was initiated in 1996 as a tool for local quality assurance in diabetes care. All diabetes outpatient clinics (DOC) and primary health care centers (PHC) are invited to participate. Patients are reported by physicians and nurses via paper forms, computer software or the Internet. In total, 75.551 patients were reported in 2003 whereof 54.213 were considered DM2 (10.547 in DOC, 43.666 in PHC).
**Results:** Mean BMI increased from 1996 to 2003 (28.3 ± 4.8; 29.1 ± 5.0; p<0.001). Mean BMI at diabetes onset increased from 28.6 ± 5.1 in 1996 to 29.7 ± 5.5 in 2003 (p<0.001) but the age at diabetes onset did not change significantly (62.7 ± 12.9; 62.4 ± 12.5; n.s.).

Hypoglycemic treatment and DM2 duration (percent; N=50.202)

| Duration | 1–4 | 5–9 | 10–14 | 15–19 | ≥20 years |
|---|---|---|---|---|---|
| Insulin | 9.8 | 16.4 | 27.5 | 38.4 | 50.3 |
| Ins+OHA | 5.4 | 16.7 | 27.6 | 29.6 | 24.4 |
| OHA | 40.5 | 48.9 | 35.7 | 25.3 | 18.3 |
| Diet | 44.4 | 18.0 | 9.0 | 6.6 | 6.2 |

Overall, in 2003 19.8% were treated with insulin only, 15.3% insulin and OHA, 39.1% OHA only and 25.8% diet only (N=54.213). 69.1% were on metformin, 48.8% on SU, 1.4% on alpha-glucosidase inhibitors, 3.6% on thiazolidindiones and 5.8% on repaglinide (n=10.526).
In 2003 still 23.7% of the patients had HbA1 c >6.0% (DCCT), 26.0% BP >130/80, 63.0% LDL >2.6, 43.7% TG >1.7, 29.3% HDL <1.1, 54.0% BMI >28, 13.9% were smoking, and 17.1% had microalbuminuria or 6.9% nephropathy. Thus, 66.3% of DM2 exhibited the metabolic syndrome (WHO definition; although WHR were not reported), while 65.4% percent received antihypertensive treatment, 37.8% lipid lowering drugs, and 36.0% ASA.
**Conclusion:** Although the standard of diabetes care has improved 1996–2003, a large proportion of DM2 patients still are exposed to a high risk for macro- and microvascular complications, according to NDR. BMI is slowly increasing but the age at DM2 onset has not decreased. It is crucial that risk factor treatment in DM2 is further improved and treatment goals are met.

# PS 2

# Metabolic syndrome around the world

## 272

**The metabolic syndrome and incident diabetes: assessment of four suggested definitions of the metabolic syndrome in a Chinese population with high post-prandial glucose**

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**Aims:** To assess the sensitivity and specificity of the four definitions of the metabolic syndrome for incident diabetes in both men and women.
**Methods:** The screening survey for type 2 diabetes was conducted in 1994. A follow-up study of 627 high-risk non-diabetic individuals at baseline was carried out in 1999 in Beijing area. With 70 male cases and 76 female incident cases of diabetes, four definitions of the metabolic syndrome based on the NCEP, WHO, EGIR and AACE recommendations were compared.
**Results:** The metabolic syndrome based on all four definitions identified men at a 3.7- to 4.5-fold and women at a 1.6- to 2.8-fold high risk for developing diabetes during 5-year follow-up. The AACE definition had the highest sensitivity for predicting diabetes (Men: 0.61; women: 0.58) and least specificity (Men: 0.71; Women: 0.70). The WHO definition identified 53% of male and 42% female incident diabetes. The NCEP definition in which adiposity was defined as waist girth >102 cm was the most insensitive detecting only 27% of incident diabetes in men although it was the most specific (0.91). The EGIR definition identified the least female cases (28%) and less male case (28%) of incident diabetes but it is quite specific (women: 0.87; men: 0.91).
**Conclusions:** Further studies on definition of the metabolic syndrome should focus on the potential ethnic differences in insulin resistance and anthropometric indicators for obesity.
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## 273

**Epidemiologic survey of the metabolic syndrome among aged 20–74 years adults in Qingdao midtown Zhanshan community**

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**Background and aims:** To estimate the prevalence of metabolic syndrome and its components among the dwellers in Qingdao midtown zhanshan community.
**Materials and methods:** A cross-sectional population based survey for metabolic syndrome was performed between April through June 2002. A total of 3000 eligible subjects were invited to participate in the survey, with an overall response rate of 87.8%, including 976 men and 1658 women. Questionnaire and examination including height, weight and blood pressure were available. After fasting at least 10 hours, all participants received a standard 75-g oral glucose tolerance test (OGTTs) in survey day, excepte those diagnosed DM before. Fasting blood specimens were collected for measurement serum total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and glucose. Complete biochemical and questionnaire data for 720 males and 1303 females were included in the analysis.
**Results:** The age-standardized prevalence of metabolic syndrome, abdominal obesity, obesity, diabetes, impaired glucose regulation, hypertension, high blood TG and low HDL-C were 11.6%, 32.1%, 11.3%, 10.0%, 12.0%, 28.4%, 24.3% and 3.9% respectively by WHO definition (1999). The prevalence of these diseases was increased with aging ($p<0.01$). The prevalence of these features of metabolic syndrome were similar for men and women aged ≥55 years, while men aged 20–55 years had higher prevalence of abdominal obesity, hypertension, high blood TG than women with the same age. About two-thirds individuals of this community had one or more metabolic disorders and one-third individuals with two or more metabolic disorders.
**Conclusion:** The results from a epidemiologic study of dweller in Qingdao show that the metabolic syndrome and metabolic disorders are highly prevalent. The large numbers individuals older than 45 years with metabolic disorders may have important implications for the health intervention care.

## 274

**Obesity and the metabolic syndrome in Hispanic and non-Hispanic white populations**

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**Background and aims:** The relationship between obesity and the metabolic syndrome (MetS) is not well studied in Hispanic populations. Obesity may be a central metabolic disorder responsible for the higher prevalence of the MetS in Westernized societies. Therefore, our aim was to analyze obesity and MetS prevalence among Hispanic and non-Hispanic white populations.
**Materials and methods:** Cross-sectional analysis of subjects aged 30 to 70 years from Spain (n = 2874), Peru (n = 565), Mexico City (n = 2282), and San Antonio, Texas. In Peru, the study targeted only Peruvian Mestizos; in San Antonio, only Mexican Americans (SAMA) (n = 2230) and non-Hispanic whites (SANHW) (n = 1238). We used the National Cholesterol Education Program (NCEP) definitions of obesity and the MetS.
**Results:** MetS prevalence was lower in men than in women in Peru (16.3 vs. 24.8%, p = 0.044), Mexico (36.2 vs. 62.0%, p <0.0001), SAMA (36.3 vs. 40.2%, p = 0.007), and Spain (20.1 vs. 28.7%, p <0.0001), but not in SANHW (28.1 vs. 25.9%, p = 0.187). Among men, obesity was more prevalent in SAMA (31.8%) than in Peru (20.1%, p = 0.002), Mexico (17.8%, p <0.0001), and Spain (24.3%, p <0.0001). However, obesity was similar in SAMA and SANHW men (31.8 vs. 34.3%, p = 0.257). In comparisons with SAMA men, MetS prevalence was lower in men from Peru (OR = 0.32, 95% CI: 0.20–0.52), Spain (OR = 0.44, 95% CI: 0.36–0.53), and SANHW (OR = 0.74, 95% CI: 0.59–0.93), and was similar in men from Mexico (OR = 1.00, 95% CI: 0.82–1.21). Among women, obesity was more prevalent in SAMA (62.8%) than in SANHW (40.7%, p <0.0001), similarly prevalent in SAMA and Peru (63.9%, p = 0.508), and less prevalent in SAMA than in Spain (66.3%, p = 0.048) and Mexico (81.6%, p <0.0001). In comparisons with SAMA women, MetS prevalence was lower in women from Peru (OR = 0.45, 95% CI: 0.33–0.60), Spain (OR = 0.55, 95% CI: 0.47–0.65), and SANHW (OR = 0.45, 95% CI: 0.36–0.56), and was greater in women from Mexico (OR = 2.42, 95% CI: 2.05–2.85).
**Conclusion:** MetS prevalence is high in Mexican-origin populations. In Peru, MetS prevalence is lower than what is expected for obesity; in Mexico City, higher. In Mexico, SAMA, Peru, and Spain, obesity and the MetS are more prevalent in women than in men.
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## 275

**Accumulation of metabolic abnormalities is strongly associated with waist circumference in men and body mass index in women in an Asian population**

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**Background and aims:** Metabolic syndrome is associated with obesity and visceral fat accumulation. To diagnose the metabolic syndrome and evaluate the visceral fat, we often use the anthropometric parameters, such as body mass index (BMI), and waist circumference (WC). Waist to hip ratio (WHR) and waist to height ratio (WHtR) are also simple anthropometric markers. In Asian people, obesity is less common and prevalence and incidence of metabolic abnormalities, such as type 2 diabetes, hypertension (HT), and dyslipidemia, are higher than European population with similar BMIs. In this study, we aimed to evaluate which anthropometric parameters associated with obesity has stronger association with metabolic syndrome in a Japanese population. We also evaluate the difference by gender in the relationship between metabolic syndrome and the indices of obesity.
**Materials and methods:** The subjects of this study were 4,557 Japanese employees (2,935 men and 1,622 women) in a metal-products producing factory, aged between 35 to 59 years. All of the subjects were examined by anthropometric measurements when they underwent their routine annual

medical check-up. Height, weight, WC and blood pressure were measured and BMI were calculated. HT, high triglyceride (HTG), low HDL cholesterol (LHDL), high fasting plasma glucose (HFPG) were defined by the guideline of National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III), and we defined the metabolic syndrome (MS) as two or more these abnormalities were observed in the same subject. Multiple logistic regression analysis was used to calculate age adjusted rate ratio (RR) of the prevalence of MS for 1 standard deviation increase in each anthropometric parameter.
**Results:** 1) The prevalence of HT, HTG, LHDL, HFPG and MS are 37.9%, 25.8%, 13.3%, 8.9%, 22.6% in men, 20.4%, 6.3%, 6.2%, 4.1%, 9.1% in women, respectively. 2) In men, the strongest association was observed with WC for HT, LHDL, with WHtR for HTG, and with WHR for HFPG. 3) In women, the strongest association was observed with BMI for HT, with WC for LHDL, HTG, with WHR for HFPG. 4) Age-adjusted RRs of the prevalence of MS elevated most strongly by WC in men (RR 1.94; 95% CI 1.77–2.14), and by BMI in women (RR 1.90; 95% CI 1.62–2.23). 5) When BMI and WC were included in the models simultaneously, WC showed an independent association with MS in men (RR 1.65; 95% CI 1.39–1.96). On the other hand, BMI showed an independent association with MS in women (RR 1.53; 95% CI 1.19–1.98).
**Conclusion:** The prevalence of MS showed the strongest association with WC in men, and BMI in women. WC in men and BMI in women should be considered more importantly for screening and making guidelines for metabolic syndrome in Asian people.

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**The prevalence of hyperinsulinemia in Polish rural and urban population: Polish multicenter study on diabetes epidemiology (PMSDE)**
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**Background and aims:** The aim of the study was to assess and to compare the prevalance of hiperinsulinemia in Polish rural and urban population
**Materials and methods:** 12000 randomised subjects aged 35–75, participants of the Polish Multicenter Study on Diabetes Epidemiology were invited to the study. In 3317 (Krakow and Lublin) and in 735 participants (Lublin rural region) antropometric and biochemical, including glucose and insulin OGTT examinations were performed. All analyses were performed in BMI (Lean-BMI below 25 kg/m$^2$; overweight-BMI between 25 kg/m$^2$ and 30 kg/m$^2$; obese-BMI above 30 kg/m$^2$) and glucose tolerance categories (normal (NGT), impaired glucose tolerance (IGT) and newly diagnosed diabetes mellitus type 2 (DM2).
Insulin-resistance was defined as the highest quartile of the insulin distribution in BMI groups, assessed for NGT population
**Results:** The mean value of fasting insulin was higher in urban than in rural population (9.9 ± 7.32 vs 8.15 ± 5.99 ($p<0.001$). The mean BMI was higher in rural than in urban population (29.12 ± 5.78 vs 28.17 ± 4.86 ($p<0.001$)). In all BMI and glucose tolerance categories the prevalence of hiperinsulinemia was from 1.8 to 3.4 times higher in urban than in rural population ($p<0,05$). The highest prevalence of hiperinsulinemia was observed in lean, overweight and obese DM2 urban subjects (34.2%, 42.1% and 49.8% ($p<0.05$). The lowest prevalence of hiperinsulinemia was observed in NGT rural population despite of BMI category (13.9%, 14.1%, 10.5% ($p<0.05$)). In obese urban subjects fasting insulin was higher than in obese rural population (11.7 ± 7.5 vs 8.4 ± 5.6) ($p<0.001$)).
**Conclusion:** The prevalence of hiperinsulinemia is higher in urban than in rural polish population despite of BMI and glucose tolerance category. The observed difference might be explained by the higher physical activity of the rural population.

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**Triglycerides is the most predictable factor of metabolic syndrome in rural Korean adults-a population study using modified ATP III criteria**
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**Background and aims:** To elucidate the prevalence and clinical characteristics of metabolic syndrome defined by the modified ATP III criteria in middle aged Korean rural people.
**Materials and methods:** Population based, cross-sectional study including 4737 participants (1984 males, 2753 females), over the age 40 was conducted at rural area in Korea. The survey provided data on anthropometric, biochemical & various questionnaires including life style. Metabolic syndrome (MetS) according to modified ATP III criteria was defined if three or more of the following criteria were satisfied: 1) Abdominal obesity; Waist circumference (WC) in men > 90 cm and in women > 80 cm. 2) Hypertriglyceridemia; ≥ 150 mg/dl. 3) Low HDL cholesterol; < 40 mg/dl in men and < 50 mg/dl in women. 4) High blood pressure; ≥ 130/85 mmHg. 5) High fasting plasma glucose; ≥ 110 mg/dl. We applied Asian Pacific Region (APR) criteria for abdominal obesity instead of ATP III criteria (WC in men > 102 cm and in women > 88 cm). Insulin resistance was analysed by HOMA-IR.
**Results:** Mean age of study population was 62.8 ± 10.4 years and mean body mass index (BMI) was 24.3 ± 3.3 kg/m$^2$. Overall prevalence of MetS was 32.1%(20.8% in men, 40.3% in women). The prevalence of MetS for each age group in men was as follows: age 40–49 (23.9%), 50–59 (21.7%), 60–69 (21.0%), and over 70 (17.9%). In women: age 40–49(28.7%), 50–59 (40.1%), 60–69 (42.0%), and over 70 (43.8%). Using ATP III criteria, the prevalence of MetS in men was 13.6% and 29.2% in women. Prevalence of abdominal obesity was 23.8% (men) & 57.2% (women) by APR criteria. The degree of increment of HOMA-IR & fasting insulin levels depended on the number of components of MetS. Relative risk of MetS increased as HOMA-IR & fasting insulin levels did. Interestingly, in men, the prevalence decreased with aging, but not in women with consistent increment. Among various metabolic risk factors, age, triglycerides, fasting glucose & systolic blood pressure were well correlated with the prevalence of MetS. Triglycerides was the most predictable factor for MetS by step-wised multiple logistic regression analysis.
**Conclusion:** The prevalence of metabolic syndrome in Korean adults was somewhat different from other previous studies, especially in men. Triglycerides was the most predicatable factor for MetS in Korean adults.
*This work was supported by 2003 Korea Health Promotion Research Program.*

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**Gender differences in the association between westernisation and metabolic risk among Inuit in Greenland and Inuit migrants in Denmark**
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**Background and aims:** The Inuit have gone through an accelerated process of westernisation especially since 1950. Primarily because of the dietary transition, westernisation is expected to influence the Inuit's metabolic risk in a negative way with respect to cardiovascular risk. The aim was to analyse metabolic risk factors among the Inuit of Greenland and Inuit migrants in Denmark and their relation to westernization.
**Materials and methods:** From 1999 to 2002, 1573 adult Inuit participated in a health survey in Greenland and Denmark. The examination included a 75g OGTT. BMI, waist circumference, and blood pressure were measured. P-glucose, s-insulin, lipids and urine albumin/creatinine ratio were analysed. The participants were categorised according to degree of westernisation based on current place of residence and proficiency in the Danish language.
**Results:** Among women westernisation was accompanied by a decrease in mean levels of waist circumference and an increase in HDL cholesterol and blood pressure. The prevalence of impaired glucose metabolism (IGM) was similar among migrants and Inuit in Greenland, and the prevalence of the metabolic syndrome decreased with westernisation and migration. Family history of diabetes and low education was associated with the metabolic syndrome. Among men westernisation was associated with a slight increase in waist circumference and BMI and an increase in blood pressure, whereas

no association was seen between westernisation and lipids or IGM. The prevalence of the metabolic syndrome increased with westernisation, and sedentary lifestyle was a significant predictor of the metabolic syndrome among men.
**Conclusion:** The effect of westernisation on metabolic risk showed important differences for men and women. For men the lack of physical activity due to a decrease in subsistence hunting and fishing seems to increase the metabolic risk, whereas for women the beneficial effect of higher education with westernisation is associated with a more favourable risk profile.

| | | Greenland | | | Denmark | |
|---|---|---|---|---|---|---|
| | *Age-adjusted* | Least westernised | Inter-mediate | Most westernised | Migrants | p |
| MEN | IGM % | 39.5 | 40.3 | 32.3 | 38.2 | *0.57* |
| | Metab. syndr. % | 16.7 | 14.3 | 22.1 | 25.4 | *0.049* |
| WOMEN | IGM% | 40.7 | 36.1 | 24.8 | 32.6 | *0.09* |
| | Metab. syndr. % | 23.4 | 23.3 | 14.0 | 15.7 | *0.03* |

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**Prevalence of the metabolic syndrome in Denmark based on the three definitions - The Inter99 study**
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**Background and aims:** In recent years several definitions of the Metabolic Syndrome has been proposed. The main difference between the three definitions is whether to include diabetes and glucose intolerance as well as measures of insulin resistance. The aim of the present study is to examine the age specific prevalence of the Metabolic Syndrome in a Danish population, and to compare the three proposed definitions (WHO, NCEP, EGIR) with respect to prevalence and cardiovascular risk.
**Materials and methods:** The population based Inter99 study included 13.016 individuals (30~60 years) randomly selected from Copenhagen County. The examination included a 75g OGTT, BMI, waist circumference, waist-to-hip ratio, and blood pressure. Plasma glucose, serum insulin, lipids and urine albumin/creatinine ratio were measured. The absolute risk of developing ischemic heart disease was calculated by the PRECARD® program.
**Summary and Results:** The population based Inter99 study included 13.016 individuals (30~60 years) randomly selected from Copenhagen County. The examination included a 75g OGTT, BMI, waist circumference, waist-to-hip ratio, and blood pressure. Plasma glucose, serum insulin, lipids and urine albumin/creatinine ratio were measured. The absolute risk of developing ischemic heart disease was calculated by the PRECARD® program. The participation rate was 52.5% (n=6784). Individuals with known diabetes and without complete data were excluded (n=6271). Age and gender prevalence of the metabolic syndrome according to three definitions is shown in the table.

| Gender | Age | n | WHO % (95% CI) | NECP % (95% CI) | EGIR % (95% CI) |
|---|---|---|---|---|---|
| Men | 30 | 145 | 13.8 (8.6-20.5) | 13.1 (8.1-19.7) | 13.8 (8.6-20.5) |
| | 35 | 298 | 17.8 (13.6-22.6) | 12.4 (8.9-16.7) | 15.4 (11.5-20.0) |
| | 40 | 587 | 22.0 (18.7-25.5) | 17.9 (14.9-21.2) | 17.2 (14.2-20.5) |
| | 45 | 675 | 29.6 (26.2-33.2) | 22.1 (19.0-25.4) | 19.4 (16.5-22.6) |
| | 50 | 668 | 34.1 (30.5-37.9) | 23.8 (20.6-27.2) | 19.3 (16.4-22.5) |
| | 55 | 509 | 41.1 (36.8-45.5) | 27.5 (23.7-31.6) | 20.2 (16.8-24.0) |
| | 60 | 243 | 40.3 (34.1-46.8) | 27.6 (22.1-33.6) | 21.0 (16.0-26.7) |
| | Total | 3125 | 30.0 (28.4-31.6) | 21.6 (20.2-23.1) | 18.6 (17.2-20.0) |
| Women | 30 | 163 | 9.8 (5.7-15.5) | 7.4 (3.9-12.5) | 9.2 (5.2-14.7) |
| | 35 | 349 | 5.4 (3.3-8.4) | 8.0 (5.4-11.4) | 5.4 (3.3-8.4) |
| | 40 | 643 | 8.9 (6.8-11.3) | 9.6 (7.5-12.2) | 8.2 (6.2-10.6) |
| | 45 | 637 | 12.7 (10.2-15.6) | 13.3 (10.8-16.2) | 10.8 (8.5-13.5) |
| | 50 | 651 | 14.9 (12.3-17.9) | 15.7 (13.0-18.7) | 13.5 (11.0-16.4) |
| | 55 | 480 | 21.9 (18.3-25.8) | 21.3 (17.7-25.2) | 14.8 (11.7-18.3) |
| | 60 | 223 | 18.4 (13.5-24.1) | 18.8 (13.9-24.6) | 11.7 (7.8-16.6) |
| | Total | 3146 | 13.2 (12.1-14.5) | 13.8 (12.6-15.0) | 10.8 (9.8-12.0) |

The prevalence increased with age using the WHO and NCEP definition, but not with the EGIR definition. Using kappa statistics, There was a moderate agreement between two definitions WHO and NCEP ($\kappa$=0.61), and WHO and EGIR ($\kappa$=0.58) and poor agreement between EGIR and NCEP ($\kappa$=0.40). The cardiovascular risk profile in individuals diagnosed by the WHO, NCEP or EGIR definitions, respectively, was: Systolic blood pressure (mean) 143, 142, 141 mmHg. Diastolic blood pressure (mean) 91, 91, 90 mmHg. Total Cholesterol (mean) 6.0, 6.0, 5.9 mmol/l. LDL cholesterol (mean) 3.9, 3.8, 3.8 mmol/l. BMI (mean) 30.4, 31.0, 31.0 kg/m$^2$. Daily smokers 33, 38, 30%. IHD risk score 12.3, 12.0, 9.1.
**Conclusion:** This study revealed that the prevalence of the metabolic syndrome in Denmark is high. Furthermore the prevalence was highest using the WHO definition. And the prevalence increased with increasing age. The cardiovascular risk profile was more unfavorable in individuals diagnosed with the WHO definition compared with individuals diagnosed by the NCEP or EGIR definition.

# PS 3

# Risk factors of metabolic syndrome and its complications

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**Prolonged television viewing increases risk of the metabolic syndrome**

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**Background and aims:** Although studies suggest that television (TV) watching increases risk, and physical activity decreases risk of obesity and type 2 diabetes, associations between both TV and physical activity and the metabolic syndrome are not well understood. We have investigated the cross-sectional relationships between both TV time and physical activity and the metabolic syndrome in Australian adults (age ≥ 25 yrs).
**Materials and methods:** Measures of 2-hour glucose, fasting triglycerides, fasting HDL-C, waist/hip ratio, BMI and blood pressure were obtained in 11,247 adult participants examined in the Australian Diabetes, Obesity and Lifestyle study (AusDiab) during 1999–2000. Fasting insulin was measured in participants aged ≥ 35 years (n = 7,982) to allow determination of the metabolic syndrome based on the WHO definition. Physical activity time was assessed by standard one week recall items; TV viewing was assessed as reported time spent watching TV and/or videos during the previous week.
**Results:** The prevalence of the metabolic syndrome was 21% (95% CI: 18.3, 23.4). After adjustment for age, sex, education, parental history of diabetes, cigarette smoking, physical activity and dietary covariates, TV viewing time was positively associated with an increased likelihood of the metabolic syndrome. The odds ratios (ORs) across increasing categories of TV (0–1.9, 2–5.9, 6–19.9, 20–40, > 40 hrs/week) were 1.0, 1.2, 1.4, 2.2, & 2.4 (P for trend < 0.001). After adjustment for the main confounders and TV time, the ORs for the metabolic syndrome across increasing categories of physical activity (0, 0.1–2.49, ≥ 2.5 hrs/wk) were 1.00, 0.98 & 0.73 (P for trend < 0.001). TV watching was positively associated with the metabolic syndrome components - diabetes/insulin resistance, obesity, hypertension and dyslipidaemia (all P < 0.05), but not microalbuminuria. With the exception of hypertension, physical activity was inversely associated with all the metabolic syndrome components (all P < 0.05).
**Conclusion:** These findings show a deleterious effect of TV time and a protective effect of physical activity on risk of the metabolic syndrome in adults. Population strategies to reduce the risk of developing the metabolic syndrome should focus not only on increasing physical activity, but also on reducing sedentary behaviours such as TV viewing time.

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**Adiposity and components of the metabolic syndrome in children: results of the Fleurbaix-Laventie Ville Santé Study**

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**Background and aims:** Adiposity is associated with increased cardiovascular morbidity and mortality in adults, but the cardiovascular consequences of excessive fat stores in children have been poorly investigated. One reason is that there are few indicators of cardiovascular disease in children. Metabolic syndrome is a clustering of clinical and metabolic abnormalities that are related to cardiovascular disease. We therefore looked for an association between increased adiposity and the prevalence of metabolic syndrome in 503 children and adolescent recruited in the general population to participate in a longitudinal study of the determinants of change in adiposity.
**Materials and methods:** Components of metabolic syndrome were high waist circumference, elevated triglycerides, low HDL-cholesterol, hypertension and high fasting glycemia. An abnormal value was defined as a value at or above the 75th percentile for waist circumference, triglycerides, blood pressure and fasting glucose, and at or below the 25th percentile for HDL-cholesterol. Presence of metabolic syndrome was defined as 3 or more abnormal values. Skinfolds (2 peripheric and 2 troncular) were measured in all children and a description was made according to quartiles of skinfolds (Q1, Q2, Q3, Q4) as a marker of adiposity. Subjects were 514 children (259 boys and 255 girls) aged 8–18, in whom the prevalence of overweight according to IOTF definition was 10%. Complete data was obtained in 503 children (256 boys and 247 girls).
**Results:** Among the 503 children in whom complete information was available, 15.4% presented a metabolic syndrome. Prevalence was 14.7% among boys and 16.1% among girls.
Increase of skinfolds was associated with a significantly higher prevalence of high waist circumference and hypertriglyceridemia in both genders, and a higher prevalence of low HDL-cholesterol only in girls (see table). The prevalence of metabolic syndrome was related to quartiles of skinfolds both in boys (Q1: 7.9%; Q2: 7.8%; Q3: 10.8%, Q4: 32.8%, p<0.001) and in girls (Q1: 3.3%; Q2: 8.1%; Q3: 8.1%, Q4: 43.5%, p<0.001)
**Conclusion:** In a general population, 15.4% of children had metabolic syndrome (defined according to the 75th percentile of distribution of each factor). In both sexes adiposity was associated with an increased prevalence of high waist circumference, hypertriglyceridemia, and metabolic syndrome. These results indicate that even in children and teenagers, adiposity is related to metabolic abnormalities that may alter long-term cardiovascular outcome.

Components of the metabolic syndrome in children according to quartiles of skinfold thickness

| Boys | Q1 | Q2 | Q3 | Q4 | p |
|---|---|---|---|---|---|
| Waist circumference > 75th perc (%) | 6.3 | 10.9 | 17.2 | 59.4 | 0.0001 |
| Triglycerides > 75th perc (%) | 12.1 | 16.4 | 23.4 | 37.1 | 0.006 |
| HDL-cholesterol < 25th perc (%) | 17.2 | 23.0 | 14.1 | 29.0 | 0.18 |
| Blood pressure > 75th perc (%) | 29.0 | 32.8 | 38.5 | 35.9 | 0.71 |
| Fasting glycemia > 75th perc (%) | 20.0 | 16.4 | 23.4 | 27.4 | 0.49 |
| **Girls** | **Q1** | **Q2** | **Q3** | **Q4** | **p** |
| Waist circumference > 75th perc (%) | 1.6 | 4.8 | 16.1 | 71.0 | 0.0001 |
| Triglycerides > 75th perc (%) | 12.7 | 15.8 | 26.2 | 40.0 | 0.003 |
| HDL-cholesterol < 25th (%) perc | 5.5 | 19.3 | 27.9 | 45.5 | 0.0001 |
| Blood pressure > 75th perc (%) | 26.2 | 25.8 | 27.9 | 43.5 | 0.07 |
| Fasting glycemia > 75th perc (%) | 21.4 | 17.9 | 17.9 | 38.2 | 0.02 |

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**Alcohol spontaneous intake and metabolic syndrome in a French population (Fleurbaix-Laventie Ville Santé): a cross sectional study**

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**Background and aims:** Several cohort studies have demonstrated a U shaped association between alcohol intake and cardiovascular disease, but the mechanisms underlying this association remain uncompletely understood. Although several studies have investigated the link between alcohol intake and insulin sensitivity, there are few data on the prevalence of the different components of the metabolic syndrome, as defined by NCEP's ATP III criteria, according to alcohol consumption. The aim of this study was to establish the prevalence of the metabolic syndrome and its different components among a population of French adults using the NCEP's ATP III criteria
**Materials and methods:** Subjects were 295 men and 341 women aged 18.0–67.1, recruited in a general population to participate in a longitudinal study on the determinants of changes in adiposity. Metabolic syndrome was defined by the prevalence of 3 or more of the following: waist circumference > 102 cm in men and > 88 cm in women, triglycerides ≥ 150 mg/dl, HDL cholesterol < 40 mg/dl in men and < 50 mg/dl in women, SBP ≥ 130 or DBP ≥ 85, glycemia ≥ 110 mg/dl. Four classes of alcohol intake were defined: CL1: 0–1.43, CL2: 1.44–10, CL3: 10.1–30 and CL4: > 30 g/day. The prevalence of each component of the metabolic syndrome, as well as their means and standard deviations, was evaluated for the 4 classes in both genders.
**Results:** Alcohol intake was associated with an higher prevalence of hypertension in men and women, and an higher prevalence of high fasting glycemia only in men (see table). The modifications in the prevalences of high triglycerides and low HDL-cholesterol with alcohol consumption were not significant. However, mean HDL-cholesterol significantly increased with alcohol intake (men: CL1: 51 +/- 13, CL2: 54 +/- 12, CL3: 56 +/- 13, CL4: 60 +/- 18, p = 0.004; women: CL1: 62 +/- 13, CL2: 66 +/- 16, CL3+4: 69

+/- 18, p = 0.007). The overall prevalence of the metabolic syndrome was similar in all classes, both for men (CL1: 8.3%, CL2: 9.1%, CL3: 9.4%, CL4: 10.7%) and for women (CL1: 8.7%, CL2: 8.3%, CL3+4: 12.9%)
**Conclusion:** Both in men and in women, the relation between alcohol intake and components of the metabolic syndrome varies according to the component considered. As a result, the prevalence of the metabolic syndrome is unmodified with alcohol intake. These results do not support the hypothesis that the lower prevalence of cardiovascular disease associated with moderate alcohol consumption is explained by a lower prevalence of the metabolic syndrome

Prevalences of different components of the metabolic syndrome according to classes of alcohol intake

| Men | Class 1 | Class 2 | Class 3 | Class 4 | p |
|---|---|---|---|---|---|
| High waist circumference (%) | 8.3 | 15.6 | 16.8 | 13.1 | 0.63 |
| High triglycerides (%) | 14.3 | 20.0 | 20.2 | 25.9 | 0.53 |
| Low HDL-cholesterol (%) | 20.0 | 12.0 | 6.4 | 7.6 | 0.11 |
| Hypertension (%) | 41.7 | 46.1 | 47.4 | 63.1 | 0.06 |
| High fasting glycemia (%) | 0.0 | 1.3 | 3.2 | 10.1 | 0.04 |
| **Women** | **Class 1** | **Class 2** | **Class 3 + 4** | | **p** |
| High waist circumference (%) | 17.4 | 15.4 | 25.7 | | 0.17 |
| High triglycerides (%) | 8.0 | 10.3 | 15.7 | | 0.26 |
| Low HDL-cholesterol (%) | 23.2 | 14.9 | 14.3 | | 0.16 |
| Hypertension (%) | 21.7 | 36.1 | 40.0 | | 0.01 |
| High fasting glycemia (%) | 6.3 | 1.9 | 7.1 | | 0.09 |

*This study was supported by the Club des Lipidologues.*

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**Baseline and three years variation of gamma glutamyl transferase and incidence of the NCEP metabolic syndrome in the D.E.S.I.R. study**
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**Background and aims:** It has been shown in cross-sectional studies, that increased Gamma Glutamyl Transferase (GGT) activities are associated with markers of insulin resistance (waist hip ratio and plasma insulin). The objective of this study is to examine the relation between baseline GGT and the variation of GGT over 3 years and the 3 year incidence of the metabolic syndrome, in a large prospective French cohort, the D.E.S.I.R. study (Data from an Epidemiological Study on the Insulin Resistance Syndrome).
**Material and methods:** We study 1853 men and 1990 women from the D.E.S.I.R. cohort, aged 30 to 65 years, without at baseline, the NCEP metabolic syndrome (MS). The MS is defined by three or more of the following criteria: waist circumference > 102/88 cm (men/women), triglycerides >=1.69 mmol/l or treated dyslipidemia, HDL-cholesterol <1.04/1.29 mmol/l (men/women), systolic/diastolic blood pressure >=130 and/or 85 mmHg or treated hypertension, fasting plasma glucose (FPG) >=6.1 mmol/l or treated diabetes. Cases of metabolic syndrome were ascertained by treatment and a physician consultation with a biologic assessment at baseline and at 3 years. Subjects were classified according to quartiles of baseline GGT and according to a decrease (≤0) or an increase (>0) in GGT over 3 years. The relation between the incidence of the metabolic syndrome over 3 years and the quartiles of baseline or the variation of GGT was analysed using logistic regression.
**Results:** 160 subjects developed the metabolic syndrome (men: 5.3%, women: 3.1%). Over the 3 years, the GGT change correlated significantly with the change in BMI (r=0.19/0.12 men/women), FPG (0.11/0.14) and fasting insulin (0.14/0.14), a marker of insulin resistance. The increase in GGT over 3 years, after adjustment on age, was not associated with an increased incidence of the metabolic syndrome, neither in men (Odds Ratio 1.3; 95% CI:0.9–2.0; p<0.13) nor in women (1.4; 0.8–2.5; p<0.16) compared to subjects with a decrease in GGT activities. But adjusted on age, the incidence of the metabolic syndrome over the 3-year period, increased across quartiles of baseline GGT, with OR 1.0, 3.5 (1.6–7.9), 3.1 (1.4–7.3), 5.4 (2.5–12.1) in men (p<0.0001) and 1.0, 0.8 (0.3–2.3), 1.6 (0.6–4.0), 2.5 (1.1–6.0) in women (p<0.001). In men, the association with baseline GGT was still significant after adjustment on age, alcohol intake, physical activity, smoking habits, transaminase, BMI at baseline and variation of GGT (p<0.02). Compared with the first quartile of GGT, subjects in the highest quartile had an increased OR of developing the metabolic syndrome of 3.0 (1.2–7.7). This relation was still significant after additional adjustment on FPG (3.0; 1.2–7.7, p<0.02) and then on fasting plasma insulin (2.6; 1.1–6.5, p<0.05). Conversely, in women the relation disappeared after adjustment on BMI (1.2; 0.5–3.0, p<0.69) or fasting insulin (1.2; 0.4–3.1, p<0.64).
**Conclusions:** These findings suggest that an increase of GGT activity is a marker of the incidence of the metabolic syndrome in both sexes. This relation was explained mainly in women and partly in men by insulin resistance. But the 3 years variation of GGT activity was not associated with the incidence of the metabolic syndrome despite a correlation with the 3 years BMI or insulin change. GGT could be a marker of hepatic insulin resistance or of a more general process linked to insulin resistance.

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**Metabolic syndrome in individuals with impaired fasting glucose and impaired glucose tolerance**
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**Background and aims:** The metabolic syndrome (MS) is a condition characterized by the simultaneous presence of a constellation of proatherogenic clinical and biochemical abnormalities. The MS represents a strong risk factor for premature cardiovascular morbidity and mortality.
The aim of our study was to evaluate the prevalence of MS in individuals with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), to identify its components and the factors that influence its presence and evolution.
**Materials and methods:** The study subjects were 284 persons with IGT and 234 with IFG, whose main characteristics of are shown in Table 1.

Table 1 - *Main characteristics of study subjects*

| PARAMETER | IGT | | IFG | |
|---|---|---|---|---|
| Gender | M | F | M | F |
| Number | 120 | 114 | 141 | 143 |
| Waist circumference (cm) * | 109.12 ± 6.2 | 94.3 ± 5.3 | 111.4 ± 8.3 | 95.3 ± 6.2 |
| TG (mg/dL)* | 234.4 ± 28.3 | 221.9 ± 22.4 | 252.4 ± 32.4 | 238.3 ± 34.3 |
| HDL (mg/dL)* | 32.3 ± 4.6 | 39.8 ± 5.3 | 31.6 ± 4.8 | 36.8 ± 4.3 |
| Systolic BP (mmHg) * | 148.6 ± 22.5 | 146.9 ± 24.6 | 152.4 ± 24.3 | 150.8 ± 26.8 |
| Diastolic BP (mmHg) * | 93.4 ± 12.4 | 94.6 ± 14.3 | 96.7 ± 10.4 | 93.5 ± 9.8 |

*Data are means ± standard deviation.

According to The Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment Of High Blood Cholesterol in Adults- Adult Treatment Panel III (ATP III), in patients with IFG and/or IGT the diagnosis of MS was confirmed if two of the following criteria were fulfilled: waist circumference >102 cm in men and >88 cm in women, plasma triglycerides ≥150 mg/dL, HDL <40 mg/dL in men and <50 mg/dL in women, blood pressure ≥130/85 mmHg.
**Results:** The MS was found in 35.04% of the patients with IFG, in 36.62% of those with IGT and in 49.29% of individuals who presented both IFG and IGT. (Table 2).

Table 2. The prevalence of MS in individuals with IFG and /or IGT

| | IFG | | IGT | | IFG+IGT | |
|---|---|---|---|---|---|---|
| Gender | M | F | M | F | M | F |
| Number | 39 | 43 | 46 | 58 | 32 | 38 |
| Percent | 32.5 | 37.72 | 32.6 | 40.56 | 45.71 | 52.77 |

The prevalence of MS was higher in women than in men (p<0.01) and increased with age.
**Conclusion:** The prevalence of MS in persons with either IFG or IGT is threefold higher than that encountered in the general population, while in individuals with both IFG and IGT it is similar to that found in patients with type 2 diabetes mellitus. Therefore IFG and IGT should not be approached as isolated conditions because often are associated with other features of the MS that, individually and interdependently, are responsible for a substantial increase in cardiovascular morbidity and mortality.

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**Association between the metabolic syndrome and cardiovascular disease using three proposed definitions: Polish Multicenter Study on Diabetes Epidemiology (PMSDE)**

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**Background and aims:** The metabolic syndrome (MS) a cluster of insulin resistance and hiperinsulinemia, disturbed glucose and lipid metabolism, hypertension and abdominal obesity is related to diabetes mellitus (DM) development and cardiovascular disease (CVD) occurrence. Estimates of the MS and related CVD vary substantially across populations because different criteria to define the MS are used.
The aim was to assess the prevalence of the metabolic syndrome and cardiovascular disease associated to MS according to the WHO, the European Group for the Study on Insulin Resistance (EGIR) and the National Cholesterol Education Program (NCEP) expert panel definitions
**Materials and methods:** 6000 randomised subjects aged 35–75, participants of the Polish Multicenter Study on Diabetes Epidemiology, were invited to the study. In 2838 participants (1225 men and 1613 women) antropometric and biochemical, including glucose and insulin OGTT examinations were performed. History of CVD defined as present/past history of CHD and/or MI and/or stroke and/or proliferative atheroslerosis was taken.
Insulin-resistance was defined as the highest quartile of the distribution of the HOMA index (for the WHO definition) and the highest quartile of the insulin distribution (for the EGIR definition) assessed for normal glucose tolerance (NGT) population
**Results:** Among 2674 examined subjects MS was present in 38% (32% women and 45% men) in 25% (23% women and 27% men) and in 35% (37% women and 32% men) subjects according to the WHO, EGIR and NCEP definition. CVD was found in 22% vs 14% (p<0,001) in subjects with vs without MS under the WHO definition. In women CVD was present in 25% vs 13% (p<0,001) of those with vs without MS under the WHO definition (OR 1,39 (95% CI; 1,03 ÷1,86). Under the NCEP and EGIR definitions CVD was present in 23% vs 14% (p<0,001) and in 20% vs 16,5%,(p< 0,05) subjects with vs without MS. In NGT group the prevalence of CVD was higher in subjects with vs without MS only under the NCEP definition (23% vs 14% (p < 0,001)). 23% vs 8% (p<0,001) and 23% vs 14% (p<0,001) of subjects with IGT/IFG and presence of MS vs absence of MS had CVD under the WHO and NCEP definition. In patients with newly diagnosed DM type 2 the presence of MS was not related to CVD
**Conclusion:** MS defined according to the WHO, NCEP and EGIR definition was related to CVD. In NGT and IGT/IFG subjects CVD was related to MS under the NCEP definition. According to the WHO definition MS was related to CVD only in IGT/IFG. In newly diagnosed DMt2 patients MS was not related to CVD.

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**Risk of death from all-cause and cardiovascular disease (CVD) related to the different definitions of the metabolic syndrome (MS) and to individual metabolic abnormalities**

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**Aim:** To study the risk of death in subjects defined as the MS by either the European Group for Study of Insulin Resistance (EGIR) or the United State National Cholesterol Education Program (NCEP) Adult Treatment Expert Panel III, and to evaluate which of the single metabolic abnormality predicted the mortality risk independent of the MS.
**Subjects and Methods:** A total of 9032 non-diabetic subjects (4103 men and 4929 women) from 7 European cohorts were included in the current collaborative data analysis. MS was defined according to either EGIR or NCEP criteria. Cox regression analysis was made to estimate the hazard ratios for MS stratified according to each of the abnormalities of hypertension, impaired glucose tolerance (IGT), impaired fasting glucose (IFG), triglyceride >2.0 mmol/l, HDL <1.0 mmol/l and obesity by BMI >30 kg/m$^2$ or by waist circumference >102 cm for men and >88 cm for women.
**Results:** In subjects without MS, either hypertension or IGT independently predicted the risk of death (table), but no independent effect was observed for other factors. Clustering of the two with others increased the risk further for CVD death, but not for all-cause death once IGT was present. Subjects with MS had increased risk of death regardless of the definitions. Compared with subjects with non-MS, subjects with MS defined by the EGIR criteria had higher hazard ratios than that defined by the NCEP criteria given the presence of IGT or hypertension.

| | Hazard ratio (95% CI) | | | |
|---|---|---|---|---|
| **EGIR** | **<140/90** | | **≥140/90 or on medication** | |
| | Non-MS | MS | Non-MS | MS |
| Total mortality | 1.00 | 1.60(1.08–2.36) | 1.51(1.26–1.81) | 1.61(1.29–2.00) |
| CVD mortality | 1.00 | 1.69(0.80–3.55) | 1.71(1.21–2.40) | 2.90(2.00–4.19) |
| NCEP | | | | |
| Total mortality | 1.00 | 1.23(0.87–1.73) | 1.49(1.23–1.79) | 1.58(1.28–1.94) |
| CVD mortality | 1.00 | 1.35(0.70–2.60) | 1.80(1.26–2.55) | 2.47(1.70–3.57) |
| **EGIR** | **NGT** | | **IGT** | |
| | Non-MS | MS | Non-MS | MS |
| Total mortality | 1.00 | 1.32(1.08–1.61) | 1.50(1.19–1.90) | 1.43(1.06–1.92) |
| CVD mortality | 1.00 | 2.01(1.46–2.78) | 1.41(0.92–2.17) | 2.14(1.36–3.39) |
| NCEP | | | | |
| Total mortality | 1.00 | 1.19(0.99–1.44) | 1.45(1.13–1.86) | 1.46(1.12–1.92) |
| CVD mortality | 1.00 | 1.57(1.14–2.16) | 1.42(0.91–2.21) | 1.86(1.20–2.90) |

*Adjusted for age, sex, center, smoking, and cholesterol.*

**Conclusion:** Hypertension and IGT are independent risk factors for death. EGIR definition identifies subjects at higher CVD risk compared with the NCEP definition.
*This analysis has been carried out with the help of grants from Novartis Pharma AG, Basel Switzerland, from AstraZeneca R&D Mölndal, Sweden, from the Finnish Academy (grants 46558, 76502, 77618, 204274 and 205657) and from Paulon Fundation.*

# PS 4

# Epidemiology of Type 1 diabetes and LADA

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**Clinical and immunological characteristics of the polyglandular autoimmune syndrome in adult patients. Is there a gender difference?**

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**Background and aims:** Historically, PAS II was defined by the occurrence of autoimmune adrenalitis, thyroiditis and/or type 1 diabetes mellitus. Since other autoimmune diseases are frequently associated with autoimmune diseases of the endocrine system, the classification (PAS I-IV) has been questioned. In order to determine the prevalence of autoimmune diseases we retrospectively analysed the clinical and immunological features of patients with ≥2 autoimmune diseases attending the outpatient endocrine clinic between 1994–2002.
**Materials and methods:** Case records were screened for the diagnosis of polyglandular autoimmune syndrome. The patient's files were analysed and the date of diagnosis of autoimmune diseases were recorded. Biochemical and immunological investigations were noted.
**Results:** Files of 78 patients were analysed (61 females (78%) and 17 males (22%); patients age at first diagnosis: females: 36.2 ± 18.0, years, mean ± SD; males: 30.6 ± 14.7, years; p=0.22). Diagnosis of autoimmune thyroiditis was established in 73 (94%) patients (Hashimoto thyroiditis: females: 36 (59%); males: 13 (76%), p=NS; Graves' disease females: 23 (38%); males: 1 (6%), p<0.02). Thirty-two (41%) patients had type 1 diabetes (females: 39 (64%); males: 8 (47%) p=NS). Autoimmune adrenalitis occurred in 9 (12%) patients (females: 6 (10%); males 3 (18%); p=NS). Associated autoimmune diseases included vitiligo in 40 (51%) patients (females: 31 (51%), males: 9 (53%); p=NS), pernicious anemia in 17 (22%) patients (females: 14 (23%), males: 3 (18%); p=NS). The prevalence of celiac disease, alopecia and hypogonadism accounted for a total of 10 (13%) patients (females: 7 (12%); males 3 (18%); p=NS).
**Conclusion:** This data suggest that a) PAS occurs with a female to male ratio of 3.6 : 1, b) the predominant endocrine diseases include autoimmune thyroiditis and type 1 diabetes in both genders, c) Graves' disease is significantly more often associated with PAS in female patients, d) the prevalence of associated autoimmune diseases was not statistically different in male and female patients and e) vitiligo is a common and easily detectable sign in patients with PAS.

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**Hypo-responsiveness to hepatitis B vaccine in patients who developed diabetes early after vaccination**

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**Background and aims:** Universal vaccination of children during the first year of life is the most effective strategy to reduce the size of the carrier reservoir and to control the spread of HBV infection. Italy was one of the first countries to initiate a policy of universal and selective high-risk-oriented vaccination against hepatitis B. In 1991 the vaccination became mandatory for all infants and 12 year-old adolescents. The present study was performed at the first universal covering of subjects not over 24 years: 12 years after introducing mandatory vaccination in all newborns and adolescents, during their 12th year of life.
The aim of this study was to evaluate the response to HBV vaccination in type 1 diabetic patients compared to a healthy population, coming from an area, as Campania region, where viral hepatitis is endemic.
**Materials and methods:** In 2002–2003 we examined a total of 219 patients with IDDM (111M and 108F), aged between 1.3 and 24.7 years (mean 13.3 ± 4.7) and 448 healthy children (295 M and 153 F), aged between 0.9 and 24 years (mean 10.2 ± 4.7). Anti-HBsAg was performed with Immuno Assay (ECLIA-Roche Diagnostics). HBsAb titre ≤10 mUI/ml was considered negative.
**Results:** Of the 219 diabetic patients and 448 control subjects 19 (8.7%) and 25 (5.5%) respectively had no record of vaccination and were excluded from the study. Thus, the HBsAb titre was determined on 200 type 1 diabetes subjects (mean age 13.2 ± 4.7 Males 99 and Females 101) and 423 controls (mean age 10.2±4.7 Males 282 and Females 141) who were all evaluated at least 2 months after the last dose of HB vaccine. No cases of clinical hepatitis were reported either in type 1 diabetes patients or controls.
There was a significantly higher percentage of non responders in the diabetic group compared to controls both in the total population and in the populations divided by age. In particular, we have found that the time between the moment of vaccination and the onset of diabetes could have conditioned the response to vaccine; in fact, there was a clear reduction of percentage of responders (47%) in the 32 children who developed type 1 diabetes during the two years after the vaccine administration compared to percentage of responders in the control group (66.4%) (p=0,043). This very significant temporal correlation, of course, does not explain the pathogenetic mechanism of the phenomenon.
**Conclusion:** If these data will be confirmed in larger populations, they represent a useful observation to screen a particular population of diabetic children risking lack of response to vaccination. This particular class of patients with age at developing diabetes less than two years after vaccination administration must be evaluated for a vaccine booster.
The higher percentage of evasion to vaccination between patients with diabetes compared to controls requires the improvement the vaccination campaign to ameliorate the coverage.

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**Does social-economical transformation influence of incidence of Type 1 diabetes mellitus?**

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**Background and aims:** The highest dynamic of increase of T1DM incidence is noted in postcomunistic countries in last 15 yrs. This period of time comes for their social-economical transformation.
The aim of the study was to analyze the influence of some parameters, which can determine the level of hygiene, health care and economic status of examined population for the incidence ratio of T1DM in children.
**Materials and methods:** Incidence of T1DM in children aged up to 15yrs (age groups: 0–4; 5–9; 10–14) was estimated accordingly to EURODIAB criteria, between 1989–2002 in Upper Silesia region (4 mln -population). For calculation of the level of hygiene, health care and economic status the following parameters were chosen: number of salmonella diseases, taeniasis, diarrhea, and diarrhea in children aged 0–2yrs, alimentary toxicosis and neonatal mortality, average women and men life expectendancy, number of unemploymed in this period of time. These data was received from Polish Official Statistics.
Statistic analysis was performed by using Spearman test and linear regression model.
**Results:** A dramatic increase in incidence ratio of T1DM in children aged 0–14yrs was observed, being doubled in period 1989–2002 (adequate: $4{,}72/10^5$ and $15{,}20/10^5$). T1DM was diagnosed in 1046 silesian children in these years. The statistical significance of correlation to incidence ratio of T1DM was found for salomonelosis (0–14yrs: p=0.002; 0–4yrs: p=0.046; 5–9yrs: p=0.01; 10–14yrs: p=0.045) and taeniasis (0–14yrs: p=0.002; 0–4yrs: p=0.14; 5–9yrs: p=0.01; 10–14yrs: p=0.0009). The rate of these infections demonstrates the negative correlation with T1DM incidence ratio of Silesian population. The health care status parameters as decreasing ratio of neonatal mortality, increasing average life of women and men expectation correlated significantly with the increase of T1DM incidence ratio (p<0.0005 for all parameters and age groups). Economic status observated as the number of unemployments did not influence of incidence ratio of T1DM.
**Conclusion:** Hygiene and health care status of population seems to modulate its susceptibility of T1DM developing.

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**Rates of Type 1 diabetes in children continue to rise over 25 years in Yorkshire, UK**

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**Background and aims:** To determine whether the incidence of childhood Type 1 diabetes in Yorkshire, UK continues to rise and in which age groups. Also to compare incidence trends by socioeconomic status, population density and ethnic group.

**Materials and methods:** Details of 3,200 children aged under 15 years were extracted from the population-based Yorkshire Register of Diabetes in Children and Young Adults, diagnosed over a 25 year period from 1978–2003. Annual mid-year population estimates were used to calculate age-standardised incidence rates per $10^5$/yr. Rates were examined separately by age group (0–4, 5–9, 10–14) and sex. Trends in incidence were determined using log-linear regression. We also used small areal-based measures of deprivation and population density, using UK census derived indices assigned via the postcode at diagnosis, to compare incidence trends over time. The proportion of patients categorised as Asian or not was compared across the cohort.
**Results:** A significant rise in incidence rates was present for all ages combined and within each age group (0–14: 2.7%/yr $p<0.001$, 0–4: 3.2%/yr $p<0.001$, 5–9: 2.7%/yr $p=0.001$, 10–14: 2.6%/yr $p<0.001$). There was a slight indication that rates may have peaked since 1999. Male incidence trends largely mirrored those for females throughout the 25 year period ($p=0.33$). The proportion of Asians with diabetes increased from 3% at the start of the study to 7% during the most recent years (Mann-Whitney $p=0.0004$), despite the background Asian population remaining stable during this time period. No significant differences were seen in the overall rates or in the trends over time by deprivation ($p=0.42$) or population density ($p=0.71$).
**Conclusion:** There has been a significant steady rise in the incidence of childhood Type 1 diabetes in Yorkshire over the last 25 years although incidence may have peaked as seen in some Scandinavian countries. The sharp increase in the proportion of Asians with diabetes warrants careful monitoring particularly with the increasing prevalence of obesity in this ethnic minority group.

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**Prevalence of childhood Type 1 diabetes mellitus in Germany**
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**Background and aims:** There are only little data on the prevalence of Type 1 diabetes in childhood and adolescence in Germany as well as in Europe. Aim of the study was to provide actual prevalence estimates of Type 1 diabetes based on data from North Rhine-Westphalia (NRW), the largest federal state of Germany covering about one quarter of the German childhood population. At the end of 2002, the population under 15 or 20 years of age in NRW amounted to 2.88 and 3.86 million persons, respectively.
**Materials and methods:** Prevalence estimates were derived from the North Rhine-Westphalian Type 1 diabetes incidence register. Newly diagnosed diabetic cases are ascertained by three data sources, the hospital-based active surveillance system ESPED, yearly inquiries among practices, and the computer-based documentation system DPV for quality control in pediatric diabetes care. Log-linear models were used to estimate the completeness of ascertainment by the capture-recapture-method. Prevalence estimates (95%-KI) were based on Poisson distribution.
**Results:** On 31 December 2002, a total of 3,206 and 5,237 diabetic children and adolescents aged 0–15 and 0–19 years were registered in NRW, respectively. Completeness of ascertainment was 95.0% (94.1–96.0%) and 91.7% (90.7–92.7). The respective prevalences (per 100.000 children) were estimated as 111.9 (108.0–115.7) and 137.7 (133.9–141.4), implying that about 1 out of 900 and 1 out of 700 children and adolescents under 15 or 20 years of age has diabetes, respectively. The ascertainment corrected prevalences were 117.9 (114.4–121.9) and 152.4 (148.5–156.3). Prevalences in boys and girls were similar (0–14 J.: 111.4 vs. 112.4, 0–19 J.: 137.9 vs. 137.4, $p > 0.8$). Age-specific prevalences for age-groups 0–4, 5–9, 10–14 and 15–19 years were 25.0, 99.9, 194.0 und 206.2, respectively. Projected from the actual prevalence data there are in total 14,000–15,000 and 25,500–26,500 diabetic children and adolescents under 15 or 20 years of age in Germany, respectively.
**Conclusion:** The North Rhine-Westphalian diabetes register enables a valid estimation of Type 1-diabetes prevalence in childhood and adolescence. At this time about 12 and 15 out of 10000 children and adolescents, respectively, have Type 1 diabetes in Germany. Actual valid estimates of the diabetes prevalence are a basic requirement for the planning of structures for diabetes care in childhood and adolescence.

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**Concordance for diabetes in 2–20 year old twins**
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**Background and aims:** It has been demonstrated that the incidence of type 1 diabetes has been increasing throughout the 90's in Denmark as well as other European countries. There are no twin studies on concordance for diabetes in the twins born in this period and the aim of our study is to provide these estimates.
**Materials and methods:** A total of 14 700 pairs of Danish twins born from 1983 to 2000 (both inclusive) were contacted by means of a 2-page questionnaire and asked about a few diseases, diabetes included. Prevalence, pairwise and probandwise concordances were estimated.
**Results:** The response rate was app. 65%, representing 19 831 twin individuals and 155 of these answered that they had diabetes. The prevalence thus is 0.8%. Twenty pairs were monozygotic, 115 dizygotic and in two pairs it was not possible to reach a firm conclusion on zygosity. Pairwise concordances were 0.40 and 0.27 in monozygotic and dizygotic twins, respectively. Probandwise concordances, i.e. the risk of developing diabetes if one's twins has diabetes, were 0.57 and 0.42 in monozygotic and dizygotic twins.
**Conclusion:** Compared to older Danish twins born from 1953 to 1982 (both inclusive) the concordance in dizygotic twins is almost twice as high in this younger cohort. This is most probably due to environmental challenges, rendering European children more susceptible to diabetes compared to earlier cohorts.

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**Mortality in Type 1 diabetes patients with onset after age 40 years**
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**Aim:** The aim of our study was to analyze the age at onset, disease duration, age at death and cause of death in patients with type 1 diabetes mellitus (T1DM) and onset after 40 yrs.
**Materials and methods:** We designed a retrospective study of T1DM patients with onset after 40 yrs., registered in Bucharest Diabetes Center and deceased between 1943 and 1995. We analyzed 2330 cases, 1175 (50.42%) males (M) and 1155 (49.58%) females (F). All patients were treated with insulin from the diagnosis. For each patient the age at diabetes onset, disease duration, cause of death and sex were recorded. Statistical analysis was made using Epi Info v3.2 (Student t test, Mann-Whitney/Wilcoxon).
**Results:** The mean age at onset was $54.32 \pm 8.71$ yrs, at death $68.22 \pm 9.26$ yrs. and the mean survival period was $13.89 \pm 8.69$ yrs. Comparing the two sexes, we found a statistical significant difference for age at onset i.e. $55.55 \pm 8.93$ yrs (F) vs. $53.12 \pm 8.32$ yrs (M), $p<0.0001$ and for age at death i.e. $69 \pm 9.09$ yrs (F) vs. $67.46 \pm 9.36$ yrs (M), $p=0.0001$. There is no statistically significant difference for survival period i.e. $13.44 \pm 8.17$ yrs (F) vs. $14.34 \pm 9.16$ yrs (M), $p=0.053$. Comparing 1942–1950 with 1990–1995 period, we found a statistical significant decrease for the age at onset from $56.99 \pm 9.00$ yrs to $51.01 \pm 8.97$ yrs, ($p=0.0001$) and an increase of survival period from $3.65 \pm 2.16$ yrs to $20.40 \pm 12.92$ yrs ($p<0.0001$) and of age at death from $60.64 \pm 9.23$ yrs to $71.42 \pm 12.41$ yrs ($p<0.0001$). The major causes of death during the 1990–1995 period were: cardiovascular diseases (53.8%), cerebral vascular diseases (17.3%), gastrointestinal and hepatic diseases (9.6%), infectious diseases (7.7%), malignancies (5.8%), chronic renal failure (1.9%), acute diabetic complications (1.9%) and others (1.9%). Comparing 1942–1950 with 1990–1995 period, the percentage of cardiovascular and cerebral vascular diseases related deaths rose 1.8 times, chronic renal failure related deaths rose 1.46 times and gastrointestinal and hepatic diseases rose 2.9 times, while infectious diseases decreased 4.24 times.
**Conclusions:** Comparing 1942–1950 with 1990–1995 period, we found a statistical significant increase of survival period, explained both by a decrease in age at onset and an increase in age at death. Females have a significantly higher age at death then males explained by a higher age at onset, the survival period with diabetes being not significantly different between sexes. Comparing the major causes of death for the 1942–1950 and 1990–1995 periods, we found that the percentage of cardiovascular and cerebral vascular diseases related deaths rose 1.8 times, chronic renal failure related deaths rose 1.46 times and gastrointestinal and hepatic diseases rose 2.9 times, while infectious diseases decreased 4.24 times.

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**Action LADA a multinational European project in latent autoimmune diabetes in adults**

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**Background and aim:** Latent autoimmune diabetes in adults (LADA) represents a sizeable proportion of all cases of autoimmune diabetes. No data has been collected prospectively in a co-ordinated fashion from different populations in Europe and no information is available as to whether a north/ south gradient exists of this form of diabetes.
**Research design:** The Action LADA project which is supported by the European Commission is aiming to define the prevalence of LADA in 9 European countries, including, England, Northern Ireland, Denmark, Spain, Finland, Germany, France, Austria and Italy. Entry criteria includes diagnosis of diabetes between 30 and 70 years, duration of <5 years, non insulin requirement for the first 6 months since diagnosis.
**Method and results:** To date there have been 1138 samples collected. Glutamic Acid Decarboxylase (GAD) antibodies were measured centrally using an IDS validated assay. Positivity for GAD antibodies were found in 127 out of 1138 (11%) ranging from 7–20% in the different centers.
**Conclusion:** Variation in GAD autoimmunity between countries introduces the possibility to assess the geographical impact of autoimmune diabetes incidence and diabetes associated autoimmunity within Europe.
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**Prevalence and phenotype of LADA in Icelandic Type 2 diabetics**

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**Background and aims:** Type 2 diabetes accounts for 90% of diabetes in Iceland. The prevalence of diabetes in Iceland is about half the prevalence in other nordic countries and has remained unchanged from 1967 to 1991 in spite of increasing obesity of the population. The prevalence of islet cell autoantibodies in Icelandic type 2 diabetics is unknown. The aim of this research was to estimate the prevelence of Latent Autoimmune Diabetes in Adults (LADA) in Iceland, describe their phenotype and to assess their relatedness.
**Materials and methods:** Data from the Reykjavik study of the Icelandic Heart Association and hospital records were used to generate a list of known diabetics. The geneological Book of Icelanders was used to identified relatives within 6 meioses of index cases. During 1998–2001 we thus generated a registry of 950 diabetics classified according to ADA criteria, representing about 25% of type 2 diabetics in Iceland. Detailed clinical and biochemical phenotyping was carried out and antibodies to glutamic acid decarboxylase (GAD-Ab) were measured by ELISA. Relatedness of GAD-Ab positives was compared to relatedness of GAD-Ab negatives by calculating Kinship coefficient.
**Results:** 10,1% of males and 9,3% of females were GAD-Ab$^+$ (ns). The mean age of GAD-Ab$^+$ and GAD-Ab$^-$ individuals was similar (67,1 ± 10,7 v 68,0 ± 11,3; y ± SD). Overall 47 ± 9% of GAD-Ab$^+$ and 60 ± 4% of GAD-Ab$^-$ fulfilled criteria for the WHO definition of the Metabolic Syndrome (p=0,02). GAD-Ab$^-$ individuals were more overweight (BMI 29,7 ± 4,4 v 28,2 ± 5,1 p=0,02; kg/m$^2$±SD) than GAD-Ab$^+$. For those not on antihypertensive medication (n=727), GAD-Ab$^-$ had slightly higher blood pressure (SBP 146 ± 21 v 143 ± 21 p=ns & DBP 82 ± 10 v 81 ± 9 p=ns; mmHg) than GAD-Ab$^+$. For those not on lipid lowering treatment (n=897) dyslipidaemia was more evident in the GAD-Ab$^-$ (Cholesterol/HDL ratio 5,3 ± 1,7 v 4,9 ± 1,5 p=0,03 & triglycerides 2,0 ± 1,3 v 1,6 ± 0,8 mmol/l, p=0,001). For the 844 individuals not on glucose lowering drugs, a wide variation was seen in HOMA-β in the GAD-Ab$^-$ group (85,1 ± 290,8 v 80,9 ± 66,7 for GAD-Ab$^+$ p=ns) and HOMA-IR for GAD-Ab$^-$ was 6,9 ± 6,5 v 8,0 ± 12,0 for GAD-Ab$^+$; p=ns. The Kinship coefficient for GAD-Ab$^+$ (n=94) was 6,00 x $10^{-4}$ as compared to 3,93x$10^{-4}$ ± 8,3*$10^{-5}$ (p=0,008) for 500 random 94 subject samples of the GAD-Ab$^-$ group (n=856).
**Conclusion:** About 10% of Icelandic type 2 diabetics have LADA as defined by the presence of GAD-Ab. This is comparable to data from other investigators. As expected, GAD-Ab$^-$ individuals have more often features of the Metabolic Syndrome than GAD-Ab$^+$. GAD-Ab$^+$ individuals in Iceland are probably more closely related to each other than other type 2 diabetics.

# PS 5

# Screening and detection strategies in Type 2 diabetes

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**Glycohemoglobin (HbA1c) as a screen for diabetes in high risk youth**

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**Background and aims:** Adults with type 2 diabetes mellitus are likely to remain undiagnosed for years, although an increasing awareness of this problem is lessening their numbers. Now, with a marked increase in the prevalence of type 2 diabetes mellitus in teenagers, many cases may remain undiagnosed in this age group as well. A simple screening test to identify those at highest risk would therefore be a useful tool. The aim of this study was to evaluate the utility and acceptability of a fingerstick A1C test in a school-based study.
**Materials and methods:** Between the fall school semester of 2000 and the end of 2003, samples were obtained on 1297 high school students and analyzed immediately for A1C using a DCA 2000®+ analyzer. All previously non-diabetic students with an A1C ≥ 5.3% (n=357, 27.6%) were invited for a fasting 75g oral glucose tolerance test. In addition, female students with lower A1C and one or more risk factors for polycystic ovary syndrome were offered an oral glucose tolerance test.
**Results:** One hundred eighteen students with an A1C ≥ 5.3% (35.4% of girls, 30.2% of boys) participated in the glucose tolerance test as did 54 girls with a lower A1C concentration. This report is limited to non-Hispanic White and Hispanic students, who together made up 94.8% of participants. Except for one outlier (A1C=8.5%) the distribution was normal (mean=5.04%, range=3.7–6.4%). A1C was also normally distributed within each sex and ethnic group. The mean A1C was similar in non-Hispanic White and Hispanic males (5.09% and 5.11%, respectively, p=0.830) but was slightly lower in non-Hispanic White females (4.97%) than in Hispanic females (5.04%, p=0.045, controlled for age). Diabetes was not found among the 37 non-Hispanic White students or the 23 Hispanic males participating in the glucose tolerance tests. However, 4 of the 55 (7.3%) Hispanic females were diagnosed with type 2 diabetes. By contrast, none of the 35 Hispanic females who had a glucose tolerance test because of high risk factors for polycystic ovary syndrome despite having an A1C <5.3% had diabetes (difference in proportions=0.073, 95% c.i.=0.004–0.141).
**Conclusion:** Screening for unrecognized diabetes was not useful in Hispanic male or non-Hispanic White students. However, among Hispanic female students who had an AlC ≥ 5.3%, over 7% of those who had follow-up were diagnosed with type 2 diabetes. Assuming this is a representative sample of all students who participated in the A1C testing, as many as 6 more cases of type 2 diabetes remain unrecognized among female Hispanic study participants who did not elect to have a follow up glucose tolerance test. In this subpopulation, screening with a fingerstick A1C proved to be a simple and useful means of identifying those at risk and was acceptable to students, teachers and school administrators. A similar screening program could also be useful in other high-risk subpopulations of children.
*Supported by: American Diabetes Association*

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**Have ATP III criteria agreement and predictive value with insulin-resistance in people with BMI ≥ 25?**

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**Background and aims:** To compare concordance of metabolic syndrome (MS) according ATP III criteria in patients with BMI ≥ 25 vs. insulin resistance measured by homeostasis model (HOMA). To evaluate the positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity of ATP III criteria for insulin-resistance and HOMA in different levels.
**Material and methods:** 275 patients, age 44.72 ± 12.18 years old, male 151 (54.9%), female 124 (45.1%), BMI 34.2 ± 6.3, euthyroid, without previous diagnosis of diabetes or glucose intolerance, or treated with metformin, thiazolidinediones, oral hypoglycemiant drugs, hypolipidemic drugs, sibutramin and orlistat were studied.
MS was diagnosed with ≥ 3 criteria. These patient were compared with HOMA (≤ 2.10, ≥2.11, ≥2.50, ≥3.00 and ≥3.50).
Statistical analysis were made through PPV, NPV and K (Kappa) coefficient (significative p<0.05).
**Results:** Patient were clasified as overweigthed (BMI 25–29.9) n=70, obese (BMI ≥30) n=162, glucose intolerant n=18 and type 2 diabetic (OMS) n=25. See tables. Adjusted by sex and by BMI≥30 and adjusted by sex and BMI≥30 and normal glucose tolerance same results were founded with PPV, NPV, sensitivity and specificity, absence of concordance is maintained in different levels of HOMA ≥2.11.
**Conclusion:** According with K coefficient there is an absence of concordance between ATP III criteria for MS and HOMA in patients with BMI ≥25 and HOMA ≥2.11. When adjusted by sex and by BMI≥30 and normal glucose tolerance same results were founded.
When more levels of HOMA ≥2.11 less PPV and especificity were founded, the opposite occurs with NPV and sensitivity.

PPV, NPV, Sensitivity, Specificity and Kappa: ATP III vs. HOMA, n=275

| HOMA | ≤2.10 | ≥2.11 | ≥2.5 | ≥3.0 | ≥3.5 |
|---|---|---|---|---|---|
| **PPV** | 0.26 | 0.74 | 0.65 | 0.54 | 0.51 |
| **NPV** | 0.35 | 0.66 | 0.75 | 0.84 | 0.88 |
| **Sensitivity** | 0.24 | 0.63 | 0.68 | 0.73 | 0.77 |
| **Specifity** | 0.38 | 0.76 | 0.73 | 0.70 | 0.70 |
| **K coefficient** | −0.38 | 0.38 | 0.41 | 0.39 | 0.40 |
| **significance** | p=1 | p<0.0001 | p<0.0001 | p<0.0001 | p<0.0001 |

PPV, NPV, Sensitivity, Specificity, Kappa: ATP III vs. HOMA, adjusted by BMI ≥30, n=197

| HOMA | ≤2.10 | ≥2.11 | ≥2.5 | ≥3.0 | ≥3.5 |
|---|---|---|---|---|---|
| **PPV** | 0.17 | 0.83 | 0.73 | 0.61 | 0.58 |
| **NPV** | 0.51 | 0.49 | 0.63 | 0.78 | 0.82 |
| **Sensitivity** | 0.26 | 0.62 | 0.67 | 0.73 | 0.76 |
| **Specifity** | 0.38 | 0.74 | 0.70 | 0.66 | 0.66 |
| **K coefficient** | −0.32 | 0.32 | 0.36 | 0.38 | 0.39 |
| **significance** | p=1 | p<0.0001 | p<0.0001 | p<0.0001 | p<0.0001 |

Adjusted by BMI ≥30 and nomal glucose tolerance, n=162

| HOMA | ≤2.10 | ≥2.11 | ≥2.5 | ≥3.0 | ≥3.5 |
|---|---|---|---|---|---|
| **PPV** | 0.24 | 0.76 | 0.65 | 0.51 | 0.46 |
| **NPV** | 0.47 | 0.53 | 0.67 | 0.82 | 0.87 |
| **Sensitivity** | 0.26 | 0.56 | 0.61 | 0.69 | 0.73 |
| **Especificity** | 0.44 | 0.74 | 0.71 | 0.68 | 0.68 |
| **K coefficient** | −0.28 | 0.28 | 0.32 | 0.34 | 0.35 |
| **Significance** | p=1 | p=0.001 | p<0.0001 | p=0.001 | p<0.0001 |

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**Which type of diabetes is diagnosed in young subjects? Multicenter analysis in Germany and Austria based on 27 008 patients with diabetes onset during the first 3 decades of life**

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**Background:** Textbook knowledge assumes that most cases of diabetes in children, adolescents and young adults are type-1 diabetes. However, during recent years, an earlier onset of type-2 has been described, and the awareness for type-3 patients ("other specific types of diabetes") is increasing.
**Patients and Methods:** The DPV-Science-Database (March 2004) encomprises longitudinal records from 27,008 patients with diabetes onset prior to age 30 years (13,783 male, 13,225 female, mean age at onset: 8.64 years). Standardized data are recorded at 181 participating centers using the DPV software and transmitted after anonymization for centralized analysis. Patients were classified according to the ADA/WHO criteria by the centers. HbA1 c-values were measured locally and mathematically corrected to the DCCT normal range (4.05–6.05%).

**Results:** 25,834 patients (95.6%) were classified as type-1-diabetes, 306 patients (1.1%) as type-2, 712 patients (2.6%) as "other types" and 156 patients as gestational diabetes. Patients classified as type-2 were significantly older at onset (15.9 years compared to 8.3 years in type-1 patients; $p<0.0001$), predominantly female (66% compared to 48% in type-1; $p<0.0001$) and considerably overweight (mean BMI-SDS-LMS: +1.76 compared to +0.16; $p<0.0001$). HbA1 c at onset was significantly higher in T1DM (9.8 ± 3.3%) compared to T2DM (8.2 ± 2.9%; $p<0.0001$). The portion of migrant patients was slightly higher in type-2 diabetes (5.9% versus 4.6 in T1DM; $p<0.0001$). The portion of newly diagnosed patients classified as type-2 diabetes increased from 0.8% in 1995 to 2.8% in 2002 (59 patients) and 2.3% in 2003 (47 patients). The most frequent diagnosis among "other specific types of diabetes" was CF-related diabetes (106 patients, mean age at onset: 14.4 years, 71% female, BMI-SDS at onset: –1.1), followed by diabetes in trisomie 21 (63 patients, 7.1 years, 59% female, BMI-SDS: +0.4), MODY (49 patients, 10.5 years, 57% female, +0.61), other pancreatic disorders (42 patients, 8.9 years, 64% female, +0.2), Turner/Noonan syndrome (24 patients, 9.1 years, 67% female, +0.4), endocrinopathies (20 patients, 10.3 years, 70% female, –0.2) and exogenous steroid therapy (20 patients, 11.7 years, 60% female). Rarer causes of diabetes were hemoglobin anomalies (15 patients), DIDMOAD syndrome (14 patients, 36% migrants!!), malignancies (13 patients) and Prader-Willi-syndrome (7 patients, BMI-SDS +3.5, 71% female). In 314 patients, no clear diagnosis has been reached so far.
**Conclusions:** While the vast majority of children, adolescents and young adults with diabetes in Germany/Austria are still patients with type-1, more patients were diagnosed as "type-2" during recent years. Following type-1 and type-2, cystic fibrosis-related diabetes is the third most prevalent form of diabetes in Caucasian subjects during the first three decades of life. While there is a slight preponderance of males in type-1, females are predominant in most other types of diabetes.

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**The prevalence of the metabolic syndrome in patients with screen detected Type 2 diabetes and impaired glucose tolerance**

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**Background and aims:** There are different criteria for the diagnosis of the metabolic syndrome proposed by the World Health Organization (WHO) and by the Third Report of the National Cholesterol Education Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. (NCEP/ATPIII). The identification of the metabolic syndrome is important so that components of the metabolic syndrome can be managed appropriately to prevent or delay progression of associated cardiovascular risk factors. We aimed to determine the prevalence of the metabolic syndrome (determined according to the two different proposals) in patients with screen detected diabetes and impaired glucose tolerance (IGT)
**Materials and methods:** In Leicestershire 2141 subjects with at least one risk factor for diabetes attended the STAR (Screening Those At Risk) programme. Each patient attended after an 8-hour fast, gave informed consent, had a 75-g OGTT, completed a general health questionnaire and anthropometric measures were taken.
**Results:** The prevalence of diabetes was 5.1% (n=110) and the prevalence of IGT was 13.3% (n=285).
Of the 110 patients identified with diabetes 66% (n=73) were classified as having the metabolic syndrome based on both the WHO and NCEP/ATPIII proposals. The positive concordance rate was 66.4%. 80% (n=88) subjects had the metabolic syndrome based on the WHO proposal alone and 71% (n=78) by the NCEP/ATP III proposal alone. 15% (n=17) patients did not meet the metabolic syndrome for either definition. The negative concordance rate was 15.5%. Patients with diabetes who were positive only for the WHO proposal had a lower average fasting glucose, (7.2 mmol/l vs. 7.8 mmol/l) a lower BMI (28 kg/m$^2$ vs. 30.5 m$^2$) and a lower waist circumference (98.4 cm vs. 105.5 cm) compared to those patients with diabetes positive by both definitions.
Of the 285 patients identified with IGT only 37% (n=108) of patients were positive by both definitions. 69.5% (n=198) subjects were classified as having the metabolic syndrome based on the WHO proposal. 43.5% (n=124) patients were classified as having the metabolic syndrome based on the NCEP/ATPIII proposal. The positive concordance rate was 50.4%. For the WHO criteria glucose intolerance (100%) and obesity (92%) were the most common criteria met whereas for the ATP criteria hypertension or use of antihypertensive medication (62%) was most common. Patients with IGT who were positive only for the WHO proposal had a lower average fasting glucose (5.85 mmol/l vs. 5.58 mmol/l) and a lower BMI (28.6 kg/m$^2$ vs. 30.3 kg/m$^2$) and a lower waist circumference (98.9 cm vs. 104.6 cm) compared to those patients with IGT positive by both definitions.
**Conclusion:** Most patients with type 2 diabetes meet the criteria for metabolic syndrome and a significant proportion of those patients with IGT also meet the criteria. The WHO criteria identifies a greater percentage of patients with the metabolic syndrome due to the higher BP and lower glucose and obesity targets.

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**Trial of combined measurements of FPG and HbA1c in the screening test for diabetes mellitus in Japanese population in Ichihara city**

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**Background and aims:** The aim of this study was to assess the associations of the measurements of both HbA1 c and FPG levels with glucose intolerance in the screening test for diabetes mellitus in japanese subjects.
**Materials and methods:** We did a screening test of 22228 general people aged 40 and older from Ichihara-city. In the 1st. screening test, The subjects were screened for the criteria of glucosuria (positive), FPG levels (over 110 and 110 mg/dl) and/or HbA1 c levels (over 5.6 and 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.
**Results:** 1) The incidense of cases with fasting hyperglycemia (FPG: over 126 and 126 mg/dl) was 4.16% and 11.05% had high levels of HbA1 c (over 5.6 and 5.6%). 2) In the GTT study, diabetes mellitus was found in 23.5%, IGT was 34.6%, and normal was 41.9%. 3) 12 cases (11.8%) among102 subjects with both fasting hyperglycemia (FPG :over110 and 110 mg/dl) and high levels of HbA1 c (over 5.6 and 5.6%) had normal pattern in the GTT study.4) Mean HbA1 c levels of diabetes mellitus, IGT and normal were 6.087,5.578 and 5.448% ($p< 0.01$ in each group) and mean FPG levels of diabetes mellitus, IGT and normal were 125.1, 108.3 and 108.0 mg/dl in the 1st.test. Mean FPG level in the diabetes group was significantly higher than that in IGT and normal group.5) In the diabetes group, incidence of cases with normal FPG (<110 mg/dl) and normal HbA1 c (<5.6%) levels in the 1st. screening test was 22.7% and 20.9%. In addition, the subjects with both normal FPG and normal HbA1 c levels were found in only 2 cases, (1.8%). 6) In the normal critera subjects in the GTT study, cases with high HbA1 c levels (over 5.6 and 5.6%) and high FPG levels (over 126 and 126 mg/dl) were found in 54.2% and 8.9%,but only one case (0.5%) had both high levels of FPG and HbA1 c in the 1st. screening test.
**Conclusion:** Measurement of both FPG and HbA1 c is useful for the screening of diabetes mellitus, but not enough to improve the precision of screening of diabetes mellitus.

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**Population-based screening for Type 2 diabetes: a comparison of two procedures. The ADDITION-Netherlands Study**

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**Background and aims:** In the ADDITION-study (Anglo-Danish-Dutch study of intensive treatment in people with screen detected diabetes in primary care) 3000 screen detected diabetes patients will be followed for at least 5 years. In the Netherlands in 2002–2004 two different stepwise screening procedures were used. We compared the proportion of screen detected diabetics between the two procedures.
**Materials and methods:** Diabetes was defined by two abnormal glucose values (WHO criteria) on separate days, either a random blood glucose (RBG) or a fasting blood glucose (FBG) or an oral glucose tolerance test (OGTT). Capillary blood glucose values were measured with calibrated HemoCue B-Glucose analysers.
Screening procedure I (four steps): A diabetes risk questionnaire (range 0–29) was sent to all 28,826 nondiabetic patients aged 50–70 years from 48 general practices in the southwestern region of the Netherlands. Those with a score ≥ 4 were invited for RBG measurement. FBG was measured if RBG ≥ 5.5 mmol/l. Diagnosis of diabetes was established if RBG ≥ 11.1 mmol/l and FBG > 6.0 mmol/l. In case of only one diabetic value (RBG ≥ 11.1 mmol/l or FBG > 6.0 mmol/l) a venous OGTT was performed to confirm the diagnosis.
Screening procedure II (three steps): The same diabetes risk questionnaire was sent to all 20,640 patients nondiabetic patients aged 50–70 years from

38 different general practices in the same region. Those with a score ≥ 6 were invited for FBG measurement. If FBG > 6.0 mmol/l always a confirmative (capillary) OGTT was performed.
**Results:**
Screening procedure I: 10,829 individuals (38%) showed up for RBG measurement, 3203 (11%) for FBG. 282 individuals (1.0% of the invited persons) were diagnosed as diabetics.
Screening procedure II: 5,249 persons (25%) showed up for FBG measurement. In 305 persons (1.5%) an OGTT was performed. In 241 subjects (1.2%) diagnosis of diabetes was established.
The proportion of screen detected type 2 patients was significantly higher in screening procedure II compared to procedure I ($p<0.05$). The number of screen detected diabetes patients per practice varied between 0 and 15 in both procedures.
**Conclusion:** Even though the three-step screening procedure had a higher cut-off point in the questionnaire, the yield of screening for diabetes in this procedure was higher. Per practice there was a wide variation in the number of detected diabetics in both procedures. Population-based screening on WHO criteria is most effective without RBG measurement.

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**Idiopathic Type 1 or ketosis-prone Type 2 diabetes?**
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**Background and aims:** Type 1 diabetes is divided to two subgroups: the autoimmune and the idiopahic, non autoimmune form. The characteristics of idiopathic Type 1 diabetes are: acute onset with ketosis and subsequent near normoglycaemic remission without need for insulin treatment. This can be followed by another acute phase with insulin dependence not initiated by any intercurrent illness. This type is mostly found in patients of African or Japanese origin. Recently there is some debate whether this diabetes is Type 1 or rather a variation of Type 2. Objective: The evaluation of patients who were diagnosed with diabetes mellitus in our department, the onset of the disease was acute, with ketosis; proved to be autoantibody (ICA, GADA, anti-IA2) negative and C-peptide positive.
**Materials and methods:** Among 77 newly diagnosed, clinically Type 1 patients 10 proved to be autoantibody negative. Among these one patient had undetectably low C-peptide level (classic Type 1 diabetes mellitus). We included the other 9 patients in our study. The study population: 3 women and 6 men, average age 48,2 years (29–61). We evaluated the familiarity, the presenting symptoms and their duration. We registered the blood glucose, urine acetone, serum lipid, C-peptide and HbA1 c levels at onset. We evaluated the follow-up levels of the latter two. We determined the HLA types.
**Results:** 4 patient had had Type 2 diabetic relatives. We observed polyuria, polydipsia and weight loss in every patient. Blurred vision occurred in 2 cases, vulvovaginitis and balanitis in 1-1. The duration of the symptoms varied between 2 weeks and 1 year (average 6 weeks). The body mass index was between 27 and 55.5 kg/m$^2$ among the patients (average 35.4), the waist-to-hip ratio was between 0,9–1,1 (average 1,01). According to these measurements, every patient was overweight/obese. The blood glucose levels on admission were between 15,9 and 33,2 mmol/l (average 22,5). We detected ketonuria higher than 1,5 mmol/l in every patient. The HbA1 c at the diagnosis was between 10,9 and 16,6% (average 14,1%). The C-peptide level at admission was between 1,8 and 3,62 nmol/l (average 2,35 nmol/l). 2 patientes had a HLA type that made them susceptible for Type 1 diabetes, (DQ2/DR3 heterozygotes), in three cases the HLA type was indifferent for diabetes. Every patient recieved insulin therapy at onset. During the follow-up 4 patients became insulin independent, 3 needed only diet, 1 diet and metformin to maintain normoglycaemia. These patients had an increase in the C-peptide levels compared to the levels at diagnosis. We performed glucagon stimulation test in two patients. We observed a notable C-peptide response. During the follow up of max. 3 years one patient had a short hyperglycaemia with ketosis and insulin dependence not caused by intercurrent illness. In this interval the serum C-peptide level of the patient did not decrease.
**Conclusion:** (1) According to the antropologic measurements (BMI, WHR), the autoantibody profile and the time-course of C-peptide levels the idiopathic Type 1 diabetic patients (or some of them) can be classified as Type 2. (2) The idiopathic Type 1 diabetes can be found not only in the African and Japanese population, but also among the Caucasians. (3) These patients can be found among those clinically Type 1 adults who are obese and antibody negative at onset. (4) The follow-up of C-peptide levels can help the diagnosis. (5) The HLA genotyping can only be evaluated if there is no susceptibility gene for Type 1 diabetes mellitus.

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**Metabolic abnormalities related to insulin resistance justify the reduction of normal fasting glucose levels. *Brazilian Metabolic Syndrome Study (BRAMS)***
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**Background and aims:** ADA has recently altered the classification of glucose tolerance decreasing the fasting glucose levels up to 100 mg/dL to be considered as normal. This new value has been used to identify individuals with high risk to develop diabetes.We analyse the population of former normals and now considered as impaired fasting glucose as a group to compare with the previous alterations.
**Materials and methods:** Metabolic parameters in 73 individuals former classified as normals and now re-classified as impaired fasting were compared to 116 individuals with fasting glucose from 110 to 126 mg/dL and 493 normals (fasting glucose up to 100 mg/dL).
**Results:** We have not obtained differences among the groups of abnormal glucose tolerance. Individuals with new criteria of glucose tolerance have the same metabolic characteristics of the previous impaired glucose.
**Conclusion:** Fasting blood glucose over 100 mg/dL is associated to markers of insulin resistance and beta cell functional deficiente like individuals with glucose over 110 mg/dL, justifying the reduction of normal values to be considered as normals.

| | Glucose <100 | Glucose 100–110 | Glucose 111–125 |
|---|---|---|---|
| Age (years) | 35,7 ± 10,4 | 39,1 ± 10,9 | 44,4 ± 12,1 |
| Glucose (mg/dL) | 86,5 ± 7,1 | 103,2 ± 2,9 ** | 118,6 ± 3,3 ** |
| Insulin (uU/mL) | 24,7 ± 21,5 | 30,1 ± 23,9 * | 31,7 ± 24,1 * |
| HOMA-IR | 5,34 ± 4,70 | 7,90 ± 6,40 ** | 8,56 ± 6,60 ** |
| HOMA-Beta | 416,3 ± 409,6 | 275,8 ± 219,2 ** | 267,3 ± 239,5 ** |
| Waist (cm) | 105,2 ± 18,8 | 114,4 ± 17,4 ** | 113,1 ± 16,1** |
| Waist/hip | 0,88 ± 0,09 | 0,92 ± 0,09 ** | 0,92 ± 0,08 ** |
| Triglycerides (mg/dL) | 132,2 ± 74,9 | 152,9 ± 72,6 * | 163,5 ± 85,1 * |
| HDL-chol (mg/dL) | 46,5 ± 12,5 | 44,2 ± 12,9 | 43,2 ± 11,6 |
| LDL-chol (mg/dL) | 126,3 ± 46,6 | 131,6 ± 36,1 | 135,2 ± 35,7 |
| Hypertension (%) | 33 | 42 * | 57 * |

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**Italien survey on the modalities for the performance of the oral glucose torlerance test (OGTT)**
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**Background and aims:** Recently revised diagnostic criteria for diabetes mellitus and the non universal accordance on the methodology for the screening of gestational diabetes mellitus (GDM) still generate discrepancies in the execution of the OGTT. The aim of the present study was to identify the methodologies followed to perform the OGTT in different laboratories for the screening of diabetes.
**Materials and methods:** A specific 5 points questionnaire was administered to specialists of laboratory medicine working in public or private laboratories nationwide in the period from June to September 2003. Collected data were analysed at national, macro-areas and regional levels.
**Results:** In the observation period 241 questionnaires were collected covering 15 out of the 20 Italian regions. Only in 50% of the cases, laboratories performed the OGTT according to protocols defined in agreement with local reference diabetes clinics, with relevant differences between regions (59,6% in northern 42,8% in central and 21,4% in southern Italy). 87,2% of laboratories performed OGTT with 75 grams of glucose in the adults and with 1,75 g/kg in children as recommended by WHO with no differences for regional distribution. Regarding the timing of sampling only 33.2% of labs followed the WHO guidelines for the collection of samples at baseline and at 120 minutes, with a great variation between areas (36,6% north vs 7,1% south). An even higher variability was highlighted in respect to the methodology for GDM screening. 49,8% of laboratories always adopted the two steps procedure with the glucose challenge test (GCT) and successive OGTT in positive cases, 4,9% used 4 samples100 g OGTT, 1,6% 2 samples75 g OGTT, 2.1% 4 samples75 g OGTT. More than 30% of centres utilised different diagnostic schemes and amongst them 62% used individually choosen procedures, 19% used only GCT not followed by OGTT in positive cases and 18% used completely different methods. Also in the case of GDM screening the macro-areas and the regional analysis did not demonstrate a significant difference respect to the global national data. Finally only the

25.6% of laboratories referred to the WHO limits for the interpretation of the results.
**Conclusions:** A great variability has been highlighted in the performance of OGTT in the Italian Laboratories in general, possibly related to poor relationships between Laboratories and Diabetes Centres. Of particular relevance is the lack of standardization in the procedures for the screening of GDM.
*We would like to thank the SIBIOC (Società Italiana di Biochimica Clinica) - SIMEL (Società Italiana di Medicina di Laboratorio) Study Group on Diabetes Mellitus for promoting and supporting the survey.*

# PS 6

# Macrovascular complications in Type 2 diabetes

## 305

**Cardiovascular risk profile of screen detected Type 2 diabetics. The ADDITION-Netherlands study**
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**Background and aims:** In the ADDITION-study (Anglo-Danish-Dutch study of intensive treatment in people with screen detected diabetes in primary care) 3000 screen detected diabetes patients will be followed for at least 5 years. In the Netherlands in 2002–2004 a population-based stepwise screening procedure was performed. The aim of the present study was to assess the cardiovascular risk profile in patients with screen detected type 2 diabetes.
**Materials and methods:** A diabetes risk questionnaire (range 0–29) was sent home to all 28,826 non diabetic patients aged 50–70 years from 48 general practices in the southwestern region of the Netherlands. If score ≥ 4, the second step of the screening procedure, a random blood glucose (RBG) measurement, followed. Fasting blood glucose (FBG) was measured if RBG ≥ 5.5 mmol/l. In case of only one diabetic value (RBG ≥ 11.1 mmol/l or FBG > 6.0 mmol/l) a venous oral glucose tolerance test (OGTT) was performed to confirm the diagnosis. Capillary blood glucose values were measured with calibrated HemoCue B-Glucose analysers.
**Results:** RBG was measured in 10,829 subjects, FBG in 3,203. Eventually 282 new diabetics (per practice: range 0–12) were diagnosed. Baseline measurements consisted of HbA1 c, fasting blood glucose, body mass index, waist circumference, lipids and blood pressure. Preliminary results are given in table 1 (n=102). In September 2004 results of all newly diagnosed patients will be presented.

*Characteristics of screen detected diabetics (n=102)*
*Mean values (Standard deviation)*
*HbA1c* 9.0% (2.2)
*FBG* 10.2 mmol/l (3.3)
*BMI* 30.3 kg/m$^2$ (6.6)
*Waist circumference* 105 cm (11) (men) 101 cm (17) (women)
*Cholesterol* 5.6 mmol/l (0.9)
*HDL-cholesterol* 1.0 mmol/l (0.3)
*LDL-cholesterol* 3.8 mmol/l (0.8)
*Triglycerides* 2.0 mmol/l (1.0)
*Systolic blood pressure* 159 mm Hg (25)
*Diastolic blood pressure* 91 mm Hg (12)

**Conclusion:** Screen detected type 2 diabetics already have a high cardiovascular risk profile. A substantial number of them are likely to benefit from an intensive multifactorial treatment. The high HbA1 c level was striking.

## 306

**Cardiovascular risk factors in different stages of glucose intolerance: the RIAD 2- study**
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**Aim:** The relative risk of hypertension, dyslipidemia, obesity, coronary heart disease and insulin resistance was estimated in different stages of glucose intolerance (impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and newly detected type 2 diabetes) compared with normoglycemic subjects (NG).
**Methods:** When continuing the RIAD study (Risk Factors in IGT for Atherosclerosis and Diabetes), were examined 2,310 subjects with a risk factor for diabetes (age between 40–70 years) with positive family history for diabetes or having diseases of the metabolic syndrome (definition by ATP III). This study is called RIAD 2. Exclusions: known diabetes and diseases or drug-affecting glucose tolerance. All subjects underwent a 75g OGTT with measurement of plasma glucose, insulin, lipids and metabolic parameters. Participants were classified in the stages of glucose tolerance according to

the 1999 WHO/ADA diagnostic criteria. Insulin resistance (IR) was assessed by HOMA.
**Results:** In table 1 the frequencies were collected as odds between the prediabetic stages and normoglycemia. Previously in the prediabetic stages was a significant higher risk found for dyslipidemia, hypertension, obesity and IR compared with normoglycemia.
**Conclusion:** In the same degree of increasing of the glucose intolerance, was observed an raise of prevalence of cardiovascular risk factors. Previously subjects with IFG have had a significant higher risk of the existence of cardiovascular risk factors and so the ground for vascular complications will be prepared.

| | NGT-IFG | NGT-IGT | NGT-CGT | NGT-DM |
|---|---|---|---|---|
| CVD/Apoplex/MI | 1.2 (ns) | 1.6 (1.1–2.3) | 2.3 (1.6–3.3) | 1.9 (1.3–2.6) |
| Family history of diabetes | 1.3 (ns) | 1.2 (ns) | 1.2 (ns) | 1.3 (1.0–1.7) |
| Dyslipidemia | 1.7 (1.3–2.3) | 2.2 (1.7–2.9) | 3.3 (2.4–4.4) | 3.8 (2.9–4.9) |
| Hypertension | 2.0 (1.4–2.9) | 1.8 (1.3–2.5) | 3.2 (2.1–4.8) | 4.1 (2.8–5.9) |
| Obesity | 1.6 (1.3–2.1) | 1.6 (1.3–2.1) | 2.0 (1.5–2.7) | 2.9 (2.3–3.7) |
| IR | 4.6 (2.8–7.5) | 2.8 (1.8–4.6) | 7.1 (4.5–11.4) | 8.7 (6.1–12.4) |

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**Coronary heart disease in individuals with diabetes, impaired fasting and normal fasting glucose**
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**Background:** Coronary heart disease (CHD) is the leading cause of death among diabetic population.
**Aims:** This study addressed the prevalence of CHD among the subjects with impaired fasting glucose (IFG) and type-2 diabetes mellitus (T2DM) as compared with those with normal fasting glucose (NFG). The study also compared the risks for CHD related to IFG and T2DM.
**Subjects and methods:** For diabetes screening, a total of 5000 eligible (≥2oy) subjects were enlisted from the randomly selected households in Dhaka City. Of them, 4157 (m / f: 1696 / 2461) subjects volunteered for investigations. The investigations included height, weight, waist-girth, hip-girth, blood pressure and fasting plasma glucose. ECG tracing was undertaken for those volunteers – a) who were older than 30y with family history of T2DM, stroke and CHD; b) who were found to have systolic blood pressure (SBP) ≥135 or diastolic blood pressure (DBP) ≥85 mmHg or fasting plasma glucose ≥5.6 mmol/l. Diagnosis of CHD was based on – a) history of angina plus ECG-positive either on rest or on stress; 2) post-myocardial infarction (MI) with Q-wave MI or non-Q-MI; 3) diagnosis made by a cardiologist.
**Results:** Two hundred one subjects fulfilled the criteria for ECG tracing. The overall prevalence of CHD was 0.9% (men 1.1%, women 0.8%: p = 0.16). The prevalence rates of CHD were 0.2, 0.9 and 6.8%, respectively, in subjects with NFG, IFG and T2DM (chi-sq. 191, p<0.001). Using ANOVA (Scheffe), we compared the variables – height, weight, waist, hip, SBP, DBP, body mass index (BMI) and waist-to-hip ratio (WHR) among subjects with NFG, IFG and T2DM. For all variables, the subjects with NFG differed significantly from those with IFG and T2DM, whereas, no variable showed significant difference between subjects with IFG and T2DM indicating the latter two groups had similar biophysical characteristics. The prevalence rates of CHD were significantly higher in the higher quartiles of age, BMI and WHR as compared with the lower quartiles (for all, p<0.01). Logistic regression analysis showed that adjusting for age, sex and obesity (BMI and WHR in separate model, coefficient of WHR entered here), the subjects with diabetes had greater risk for CHD (OR 22.23 with 95% CI, 8.72–56.69). In contrast, though the IFG group showed higher risk it was not significant (OR, 3.01 with 95% CI, 0.59–15.45).
**Conclusions:** The prevalence of CHD was found many folds higher in subjects with diabetes. Diabetic men and women had equal risk for CHD. Though the IFG and diabetes groups appeared to own similar biophysical characteristics only the diabetes group had significant risk for CHD. Further study, taking IGT group following OGTT, may reveal whether there is any difference of CHD prevalence between subjects with diabetes and impaired glucose regulation in the study population.
*Supported by: World Health Organization, BIRDEM*

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**Risk of myocardial infarction in Type 2 diabetes in the United Kingdom**
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**Background and aims:** There is little data on risk of myocardial infarction (MI) in type 2 diabetes (T2D) separately from type 1 diabetes. The General Practice Research Database (GPRD) provides longitudinal data from a representative population of the UK. The aim was to compare the risk of MI in people with T2D and a reference population with no evidence of diabetes.
**Materials and methods:** Patients with T2D prior to 1992 and age greater than 35 and less than 90 were identified on the database. T2D was differentiated from type 1 diabetes using an algorithm based on age at diagnosis and treatment. An age and sex matched reference group of five subjects per patient, with no record of diabetes at anytime in their record was also selected. MI events were identified using OXMIS and Read Codes and defined as definite, probable and possible according to supporting evidence within two months of the code, such as hospital discharge, change in treatment, symptom or revascularisation. Any subject in the patient group or reference group with a code for MI prior to 1992 was excluded from the analysis. Analyses were run using only the definite and probable MI group and a further sensitivity analysis run including those with possible MI's. Risk estimates were adjusted for age, duration of diabetes at baseline, smoking status and sex.
**Results:** The analysis was based on 40,557 patients with type 2 diabetes and 193,763 reference subjects. The prevalence of MI was 18.1 per 1,000 person years (95% CI: 17.4, 18.7) in T2D and 7.0 (95% CI: 6.8, 7.2) in the no diabetes group. The adjusted hazard ratio (HR) for MI compared to no diabetes was 2.35 (95% CI: 2.22, 2.48). Men with diabetes were at more than twice the risk of MI compared with no diabetes [adjusted HR 2.06 (95% CI: 1.91, 2.22)], whilst the risk amongst women was almost three times greater than women without diabetes [adjusted HR 2.83 (95% CI: 2.59, 3.09)]. The risk of MI amongst smokers was 1.54 (95% CI: 1.44, 1.65) after adjusting for age, diabetes and sex. Amongst women the risk of MI associated with smoking appeared higher and was statistically different to that amongst men [adjusted HR 1.94 (95%CI: 1.72, 2.20) versus HR 1.41 (95% CI: 1.30, 1.52). The sensitivity analysis showed that including patients categorised as possible MI (having only a code for MI and no supporting evidence within two months from the code) did not alter the risk estimates.
**Conclusion:** Risk of myocardial infarction in type 2 diabetes is high. This risk is even higher for women and doubled again in women who smoke.
*This study was funded by a research grant from the British Heart Foundation.*

## 309

**Peripheral arterial disease and elevated homocysteine plasma levels are independent risk factors for premature death and coronary vascular events in elderly patients with Type 2 diabetes**
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**Background and aims:** The German Epidemiological Trial on Ankle Brachial Index (getABI) Study is an ongoing large-scale epidemiological prospective 3-year trial in 6,880 unselected patients ≥ 65 years in 344 representative practices across Germany. We aimed to assess the "real life" association between peripheral arterial disease (PAD), homocysteine (HC) level or other known risk factors with the cardiovascular (CAD) risk.
**Materials and methods:** PAD was defined as ankle brachial index (ABI) < 0.9, determined with standardized Doppler ultrasonography. Fasting plasma HC levels were measured at baseline in all patients with high-performance liquid chromatography. Diabetes was defined according to the clinical diagnosis of the physician and/or HbA1 c ≥ 6.5% and/or intake of oral antidiabetic medication and/or application of insulin. Severe CAD events (cardiac death, myocardial infarction, coronary revascularisation) during follow-up were reported by GPs.

**Results:** 1,743 patients were classified as diabetics (DIA): mean age was 72.5 ± 5.4 years, 51.4% were females, median $HbA_{1c}$ was 6.6% [5.9;7.3], median HC was14.3 µmol/l [11.2;18.2], 26.3% had PAD. One-year all-cause mortality of DIA vs. no DIA was 2.0% vs. 1.0% (Odds ratio [OR], adjusted for known risk factors: 1.9 [95% confidence interval: 1.2;3.1]). The incidence of severe CAD events was 2.8% (DIA) vs. 1.5% (no DIA; OR: 1.6 [1.1;2.3]). Patients with DIA+PAD vs. DIA/no PAD had a substantially increased risk of premature death (univariate OR: 5.4 [3.1;9.4]), or of severe CAD events (OR: 4.2 [2.5;7.0]). Patient with DIA+ high HC (>80% percentile, i.e. >19.1 µmol/l) vs. no DIA/normal HC (HC ≤ 80% percentile) were also at increased risk of death (OR: 5.6 [2.8;10.9]), or of severe CAD events (OR: 3.4 [1.9;6.2]). The highest risk was found for DIA+PAD+high HC (OR for death: 8.6 [3.4;21.7], OR for CAD events: 6.7 [2.9;15.5]).
**Conclusion:** We found a strong and independent association between elevated serum HC level, and risk for premature death and severe CAD events. An association of greater strength was found for the combination of high HC + PAD and death or CAD events. Thus, screening for high HC as well as PAD appears to be of value to identify those patients at particularly high CAD risk.
*This study was supported by an Unrestricted Educational Grant by Sanofi-Synthelabo, Berlin.*

## 310

**High-sensitivity C-reactive protein and coronary heart disease mortality in Type 2 diabetic patients. A 7-year follow-up study**
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**Background and aims:** Inflammation plays an important role in the pathogenesis of atherosclerosis. High-sensitivity C-reactive protein (hs-CRP) is a systemic inflammatory marker, and it has been found to predict future coronary heart disease (CHD) events in many prospective studies in healthy subjects already at a level > 1 mg/L. Concentration of CRP is increased in type 2 diabetes. Type 2 diabetic patients have a 2-to 4-fold increased risk for CHD. Our aim was to examine in a large prospective follow-up study whether hs-CRP predicts CHD events also in this high risk population for CHD.
**Materials and methods:** The original study population in 1982–1984 consisted of 1059 type 2 diabetic patients aged 45–64 years. Mean duration of diabetes was 8 years. Samples for hs-CRP determination in 2003 were available in 944 of them. From these patients 793 were free of myocardial infarction (MI) at baseline. CHD mortality and incidence of nonfatal MI were assessed at the 7-year follow-up study in 1990, i.e. before the era of statin treatment.
**Results:** From 944 patients 148 died from CHD and 235 had a nonfatal or fatal CHD event. Patients with hs-CRP > 3 mg/L (n=312) had a 1.5-fold higher risk for CHD death compared to those with hs-CRP ≤ 3 mg/L (n=632) (19.9% and 13.6%, respectively, p=0.013). In Cox regression analyses adjusting for age, gender, duration of diabetes, total cholesterol level, high-density lipoprotein cholesterol level, triglyceride level, smoking, hypertension, body mass index, glycosylated hemoglobin A1 and area of residence, hs-CRP was still significantly associated with CHD death rate (p=0.011). Patients with hs-CRP levels < 1 mg/L and 1.0–3.0 mg/L did not differ in their CHD death risk. There was no significant association between hs-CRP and the combined end point nonfatal or fatal MI. In those patients who were free from MI at baseline, hs-CRP level > 3 mg/L also independently predicted the risk of CHD death (p=0.011).
**Conclusion:** In this large cohort of almost 1000 type 2 diabetic patients hs-CRP is an independent risk factor for CHD death in this high CHD risk population, but only at a higher hs-CRP level (> 3 mg/L) compared to previous observations in nondiabetic populations.

## 311

**Does high sensitive CRP serve as a risk factor of cerebrovascular diseases and coronary heart diseases?**
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**Background and aims:** As chronic inflammation has been considered to be one of the causes of atherosclerosis, a parameter for its clinical evaluation has been sought. Among inflammation markers, high sensitivity C-reactive protein (hs-CRP) has become possible and it has been attracting interest as risk factor for atherosclerotic diseases. We therefore conducted measurement of hs -CRP in examinees in a fixed population under long-term follow-up and studied the correlation of hs -CRP to cerebrovascular diseases (CVD) and coronary heart diseases (CHD).
**Materials and methods:** The subjects of the present study were 13,436 individuals composed of 5,705 males and 7,731 females who were examined between October 2003 and February 2004. Their mean age was 70.0 ± 7.3 (mean±SD) years in male and 70.9 ± 8.0 years in female. In order to avoid high hs-CRP values due to active inflammatory diseases, those with WBC exceeding 10,000/µL, blood sedimentation rate exceeding 30 mm in 1 hr or those with liver dysfunction were excluded. According to detail medical history, we ascertained the presence of CVD including cerebral thrombosis and cerebral hemorrhage and CHD including acute myocardial infarction and angina pectoris.
**Results:** A marked deviation was observed in the distribution of hs-CRP especially at the lower value range. The subjects were divided into quartile in each gender (male; the first quartile, Q1: <0.42 mg/L, Q2: 0.42–0.72 mg/L, Q3: 0.73–1.38 mg/L, Q4: ≥1.39 mg/L, and females; Q1: <0.37 mg/L, Q2: 0.37–0.60 mg/L, Q3: 0.61–1.09 mg/L, Q4 ≥1.10 mg/L). 1) Significant correlation of hs-CRP to blood pressure and BMI, were demonstrated in both male and female. 2) Prevalence of CHD for male were 5.2% in Q1 of hs-CRP, 7.4% in Q2, 8.4% in Q3 and 10.0% in Q4, for female the rates were 4.9%, 5.9%, 6.0% and 7.7%, respectively. With elevation of hs -CRP the prevalence of CHD increased step wisely in both gender (p<0.001). 3) Prevalence of CVD for male were 7.2% in Q1 of hs-CRP, 6.8% in Q2, 9.9% in Q3 and 11.4% in Q4, for female the rates were 4.5%, 5.4%, 7.3% and 7.37%, respectively. The prevalence of CVD elevated significantly with increase of hs-CRP both in male (p < 0.001) and in female (p < 0.01). 4) The risk factors for CVD and CHD were analyzed by logistic regression model. Odds ratio of CVD for age and log hs-CRP were 1.04 and 1.61 in male, 1.04 and 1.45 in female, respectively, those were all statistically significant. Odds ratio of CHD for age, BMI and log hs-CRP in male were 1.03, 1.06 and 1.73, respectively. Those for age, hemoglobin A1 c and log hs-CRP in female were 1.05, 1.17 and 1.53, respectively. These odds ratios were all statistically significant.
**Conclusion:** Significant correlation of high sensitive CRP to CVD and CHD was observed, suggesting that high sensitive CRP serves as a risk factor of CVD and CHD.

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**Physical activity protects against cardiovascular mortality among middle-aged men and women with Type 2 diabetes**
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**Background and aims:** Sedentary lifestyle, hypertension, hypercholesterolemia and smoking increase cardiovascular (CVD) mortality among diabetic patients. However, the joint effects of these risk factors are not known. The aim of this study is to evaluate both single and joint associations of physical activity and conventional CVD risk factors (body mass index, blood pressure, cholesterol and smoking) with CVD mortality among diabetic patients.
**Materials and methods:** We prospectively followed 3708 randomly selected type 2 diabetic patients aged 25–74 years. Physical activity and other parameters were determined at baseline. Both single and joint effects of different levels of physical activity and other risk factors with the risk of CVD mortality were examined using Cox proportional hazard models.
**Results:** During a mean follow-up of 18.7 years, 906 died from CVD. The multivariate-adjusted (age, sex, study year, body mass index, systolic blood pressure, total cholesterol and smoking) hazard ratio of CVD mortality associated with low, moderate and high physical activity were 1.00, 0.61 (95% CI 0.52–0.73) and 0.52 (95% CI 0.44–0.62). The highest tertiles of body mass index, diastolic blood pressure, systolic blood pressure and total cholesterol, as well as current smoking were each significantly associated with an increased risk of CVD mortality, in comparison with the lowest tertiles of body mass index, diastolic and systolic blood pressure, total cholesterol, and never smoking (all P<0.05). The protective effect of physical activity was consistent in diabetic patients with any levels of body mass index, blood pressure, cholesterol and smoking.
**Conclusion:** Moderate or high levels of physical activity reduce CVD mortality among patients with type 2 diabetes. The protective effect of physical activity was observed regardless of the levels of body mass index, blood pressure, cholesterol, and smoking.
*Supported by: the Finnish Academy (grants 46558, 53585, 204274, and 205657).*

# PS 7

# Complications and treatment strategies: Type 2 diabetes

## 313

**DIG - Diabetes in Germany - a prospective study for a four years period**

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**Background and objective:** A closely monitored blood pressure, optimal blood glucose regulation and treatment of dyslipidaemia are effective measures in lowering the risk of vascular disease in diabetic patients. But which clinical practice can we find in Germany? The DIG (Diabetes in Germany) study is an epidemiological survey, collecting data on the prevalence and treatment patterns of type 2 diabetes patients in Germany for a four years period.
**Patients and methods:** A national sample involving 240 physican practices. Each physican includes 20 type 2 diabetic patients randomly (age 40–70 years). In an intermediate analysis we included the data of 2,465 patients out of 222 physican practices. The anthropometric, demographic, anamnestic and medical data of the patients were examined in five therapy groups:1 - diet, 2 - one oral antidiabetic drug, 3 - more than one oral antidiabetic drug, 4 - combination insulin and oral antidiabetic drug, 5 - insulin.
**Results:** 85.6% of all patients are hypertensive, 80.2% of these patients received antihypertensive drugs (45.45% ACE-inhibitors, 34.8% beta-blocking drugs, 26.2% diuretics, 20.9% Ca antagonists, 12.2% AT1 blocking drugs and 2.8% alpha blocking drugs), and 19.8% are hypertensive without getting drugs.Table 1 shows anthropometric, anamnestic and medical data in the five therapy groups, and table 2 shows the association with micro- and macrovascular complications.
**Conclusion:** Germany has a high number of multimorbid type 2 diabetes patients. The current data continues to show a clear association between type 2 diabetes, hypertension and micro- and macrovascular complications in elderly. The study demonstrated that the control of blood glucose and blood pressure is inadequate for the majority of diabetic patients.

Table 1 *mean (standard deviation)

| Therapy group | n | age (years)* | BMI (kg/m²)* | blood pressure* | duration of diabetes (years)* | HbA1c (%)* |
|---|---|---|---|---|---|---|
| 1 | 312 | 61.1 (8.5) | 29.6 (5.1) | 137/81 (16/9) | 3.6 (2.4) | 6.3 (0.9) |
| 2 | 568 | 61.4 (9.1) | 30.2 (5.3) | 139/82 (16/9) | 5.8 (5.6) | 7.1 (3.9) |
| 3 | 441 | 61.1 (8.5) | 30.8 (5.1) | 140/83 (17/8) | 8.3 (7.8) | 7.2 (1.2) |
| 4 | 521 | 61.5 (8.3) | 32.2 (5.7) | 140/82 (18/9) | 11.3 (8.3) | 7.5 (1.3) |
| 5 | 655 | 61.4 (8.3) | 30.0 (5.1) | 139/80 (19/10) | 12.5 (8.7) | 7.3 (1.3) |

Table 2

| therapy group | diabetic retinopathy (%) | gangrene/ neurotr. ulcer (%) | amputation of foot (%) | angina pectoris (%) | myocard. infarction (%) | PAD (%) | apoplexy (%) |
|---|---|---|---|---|---|---|---|
| 1 | 4.4 | 0.6 | 0.3 | 8.7 | 5.4 | 9.3 | 2.9 |
| 2 | 4.4 | 1.1 | 0.2 | 7.9 | 7.2 | 6.3 | 2.3 |
| 3 | 9.1 | 1.6 | 0.9 | 10.9 | 4.1 | 7.7 | 5.0 |
| 4 | 28.4 | 3.3 | 1.2 | 11.0 | 6.8 | 11.2 | 4.5 |
| 5 | 26.6 | 3.4 | 1.7 | 13.3 | 11.0 | 14.1 | 5.6 |

## 314

**Blood glucose control in Type 2 diabetic patients treated with one oral hypoglycemic agent: Results from a nationwide French Survey**

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**Background and aims:** In order to describe the metabolic control of type 2 diabetic patients (T2D) treated with one oral hypoglycemic agent we conducted a prospective nationwide epidemiological survey among 1687 random French general practitioners (GPs).
**Material and methods:** Each GP had to recruit the first 4 consecutive patients consulting over a 3-month period and treated with one oral hypoglycemic agent (OHA).
**Results:** 5369 patients (mean age 65 ± 11 years, 60% males and median diabetes duration 4 years) were included by 1396 GPs between April and September 2003 and analysed. Their characteristics were the following: 35% of patients had a BMI ≥30 kg/m², 64% were hypertensive, 24% had a macro vascular complication of diabetes and for most patients (74%) diabetes had been diagnosed by chance. At inclusion, 43% of patients had a Blood Pressure >140–80 mmHg, lipid dosage was performed in 75% of patients of whom 33% had a LDL cholesterol > 1.6 g/l or/and a TG > 2 g/l. Within the previous year, 78% of patients had 2 or more evaluations of HbA1 c and at least one measurement of HbA1 c was reported for 96% of patients overall. Based on the last available value, 50% of patients had a HbA1 c ≤6.5%, 65% a HbA1 c < 7% and 12.5% a HbA1 c over 8% (HbA1 c adjusted for a norm < 6%). In the whole population, mean and median HbA1 c were respectively 6.7 ± 1.2% and 6.5%. The most frequent initial OHA used in monotherapy were the following: sulfonylureas (58%), metformin (34%), glinids (4.5%) and alpha glucosidase inhibitor (3.5%). During the inclusion visit, the initial OHA was maintained at the same dosage in most patients (72%), and a higher dosage was prescribed in respectively 10% and 20% of the overall and the poorly controlled (HbA1 c > 8%) population. During the inclusion visit, 2 or more OHA were prescribed in respectively 8% and 20% of the overall and the poorly controlled (HbA1 c > 8%) population. A multivariate logistic regression analysis was performed to identify the predictive factors of optimal glucose control (HbA1 c ≤6.5%). The results were the following: no dyslipidimia (odds ratio: OR=1.75), no microvascular complication (OR=1.47), at least 2 dosages of HbA1 c per year (OR=1.23), OHA duration ≥ 1 year (OR=1.28), BMI < 30 kg/m² (OR=1.17) and male sex (OR=1.14).
**Conclusion:** This survey confirms the improvement in quality of care in T2D as observed in the French health care fund (CNAMTS) program. Although metabolic control is satisfactory for most of the patients treated by one OHA, improved disease management is still required in poorly controlled patients.

## 315

**Does type of onset in Type 2 diabetes mellitus have prognostic value in terms of glycaemia and diabetic complications?**

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**Background and aims:** The aim of this study was to investigate general characteristics of type 2 diabetic individuals with different symptomatology leading to diagnosis and to assess whether they differ in due course of the disease in terms of glycaemic control and diabetic complications.
**Materials and methods:** Records of 911 type 2 diabetic patients (65,1% female, age 60.9 ± 10.6 years) attending the diabetes outpatient clinic of a state hospital between 1996 and 2003 with a mean follow-up duration of 3.9 ± 2.5 years were screened for symptoms leading to diagnosis of diabetes (acute = coma, acute exacerbation; slow = subacute clinical symptoms like polydypsia, polyuria, polyphagia, weight loss etc; coincidental diagnosis = no specific symptomatology), diabetic complications and A1 c. Except for descriptives (mean±SD,%), analysis of variance was used for parametric data; likelihood ratios (LHH) with Chi-square statistics for proportions were calculated via crosstabulation.
**Results:** Patients were grouped according to symptomatology as group 1: acute onset 22.1% (n=201), group 2: slow onset 18.6% (n=169), group 3: coincidental diagnosis 59.4% (n=541). The groups differed significantly in terms of age of onset of diabetes (group 1 was youngest with 47.1 ± 10.8 years vs. group 3 with 49.6 ± 10.8 years, $p$=0.005), baseline A1 c (group 1 highest with 9.8 ± 2.9% vs. group 2 with 8.95 ± 5.0% and group 3 with 8.1 ± 2.3%, $p$=0.005) and mean A1 c during follow-up (group 1 with 8.5 ± 2.2% vs. group 2 with 7.7 ± 1.8% and group 3 with 7.7 ± 1.9%, $p$<0.0001). Likelihood of baseline retinopathy (LHH 10.9, $p$=0.004), neuropathy (LHH 11.8, $p$=0.003) and coronary heart disease (LHH 5.1, $p$=0.07) was highest in group 2. This trend continued during follow up with a higher likelihood of developing retinopathy (LHH 10.04, $p$=0.007), neuropathy (LHH 35.4, $p$<0.0001) and coronary heart disease (LHH 6.98, $p$=0.03) for individuals with slow onset of type 2 diabetes mellitus (T2DM), compared to individuals with acute onset or coincidental diagnosis.
**Conclusion:** Individuals with acute symptomatology at T2DM diagnosis were characterized by younger age and bad glycaemic profile at baseline and during follow-up which, however, was not reflected in their diabetic

complication status. Slow onset T2DM was characterized with a high prevalence of diabetic complications at baseline and during follow-up and a better glycaemic prognosis. Coincidental diagnosis without symptoms was most prevalent and characterized with female predominance, older age at diagnosis and moderate glycaemic and diabetic complication profile.

## 316

**Poor hypertension control in Greek patients with diabetes. The V.A.N.K study in primary care**

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**Background and aim:** To determine the prevalence of hypertension and the levels of awareness, treatment and control of hypertension in diabetic patients using data from VANK study.
**Material and methods:** The VANK* study is a multicenter, cross sectional survey which was carried out in Primary Care. In this analysis we studied 221 men and women (122/99) patients with type 2 diabetes. Semi-structured interviews were conducted with all participants. Blood pressure was measured by using a standard mercury sphygmomanometer with appropriate cuff size. Controlled hypertension definition was based on systolic BP <130 mmHg and diastolic BP <85 mmHg in subjects taking antihypertensive medication.
**Results:** The mean age±SD of sample is 68.8 ± 11.5 years. No statistically significant difference between men and women was found (67.8 ± 10.9 vs 68.3 ± 11.2/p=0, 7). The mean duration of diabetes±SD was 12.4 ± 6.7 yrs. The mean±SD value of blood pressure (BP) was 141.6 ± 17.4 mmHg and 81.2 ± 9.4 mmHg for systolic and diastolic BP respectively. Systolic and diastolic BP appeared to be higher in men than in women. The overall prevalence of hypertension was 194/221 (87.7%). In total, 34.1% (66/194) of hypertensive patients were not aware of having hypertension. Of those who were aware of having hypertension (N=128, 65.9%), all were treated. Among treated hypertensives, only 11 persons (11/194,5.6%) had systolic BP <130 mmHg and diastolic BP<85 mmHg and 62(38.7%) had systolic BP<140 mmHg and diastolic BP <90 mmHg. In a logistic regression analysis variables associated with hypertension were age (Wald= 12.6, p=0,0004),history of coronary heart disease (Wald= 6.5, p=0,01, RR=6.4 95%CI: 1.5–26.6) and family history of hypertension (Wald=10.6, p=0,001, RR=2.9 95% CI: 1.5–5.6).
**Conclusion:** In our study, all patients diagnosed with hypertension (N=128) received antihypertensive drug therapy, and only in 8.6% of them (11/128,) treatment was effective (B.P <130/850 mmHg). Translating our findings into clinical practice there is a need for aggressive treatment of hypertension from primary care physicians and a regular surveillance to detect developing hypertension in diabetic patients.

## 317

**Management of Type 2 diabetic patients in France: any difference among physicians? The observational epidemiologic SIMPA study**

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**Background and aims:** The observational epidemiologic SIMPA study (étude obServationnelle de la prIse en charge Médicale des liPides chez le diAbétique de type 2) was conducted in France to assess the medical management of lipid disorders in type 2 diabetic patients, either by general practitioners (GP's) or by specialists (diabetologists and cardiologists), and to evaluate the effects of clinical/biological status on their practical therapeutic attitude.
**Materials and methods:** The study was conducted from June to October 2003. A random sample of 3,329 physicians (2,514 GP's, 682 cardiologists, 133 endocrinologists) from the 22 French administrative regions participated. Each physician had to fill out a questionnaire for the first 3 consecutive type 2 diabetic patients [fasting glycaemia ≥ 7.0 mmol/l or treated with oral antidiabetic agent (OAD)]. 9,706 questionnaires were obtained.
**Results:** Reasons to consult were: usual diabetes follow-up (68.8%), management of diabetic complication(s) (29.2%), other reasons (19.3%). Among diabetic complications, cardiovascular complication (CVC) was the most frequently cited reason to consult, either in GP's practice (15.9%) or cardiologists (60.2%) or endocrinologists (10.6%). Patients' characteristics : mean age 64 ± 10 yrs, 63% men/37% women, 56.5% overweight/obese (BMI > 28 kg/m$^2$), 15.9% current smokers, duration of diabetes ≤ 1 to ≥ 20 yrs (mean 7.4 yrs), hypertension 69.8% (treated in 79.6%). Follow-up previous year: patients with full lipid profile 80%, retina exam (RE) 59%, ECG 71%,creatininemy 92%,proteinury/hematury 46%. GP's prescribed themselves the exams 90% of time (except RE and ECG: 80%), diabetologists 70% (all exams), cardiologists 20% to 30% (except ECG 70%). Diabetes treatment: none (1%), diet + physical activity (4%), OAD (91%), insulin + OAD (4%). Among treated patients: mono (54%), bi (40%), tri (6%) therapy.% of treated patients with HbA1C < 7% respectively 54%, 34% and 29%. HbA1 c was checked for 94% patients in last 12 months; median time: 1.51 months. Most recent HbA1 c: 7.3 ± 1.3% (4.1–14.8%). 44% have HbA1 c <7% with no difference if absence/presence of CVC. 47% have HbA1 c <7% according to cardiologist, 44% GPs, 42%, diabetologists. According to physicians perceptions hyperlipidemia (HLP) was present in 80.7% of patients, with no differences according to physicians' qualifications or patients' sex. They were classified by physicians as isolated hypercholesterolemia (31.0%), mixed HLP (61.6%) or isolated hypertriglyceridemia (7.4%). Mixed HLP was more frequent in patients with history of CVC (65.6 *vs* 59.5%, p<0.0001). Most recent full lipid profile control was 4.3 ± 6.4 months previously, with: total cholesterol (T-chol) 5.50 ± 1.08 mmol/l, LDL-C 3.28 ± 0.93 mmol/l, HDL-C 1.32 ± 0.36 mmol/l, triglycerides (TG) 1.91 ± 1.07 mmol/l. All mean lipid values were significantly higher in GPs patients and lower in endocrinologist's patients, cardiologist's patients being intermediate. T-chol and LDL-C were significantly lower in patients with CVC history.
**Conclusions:** Based on what previous studies showed, significant progress in diabetic patient medical management have been realized in France, with slight differences on lipid management between physician's type.

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**What should be the target FBG to achieve the HbA1c goal of 7% in Type 2 diabetic patients treated with basal insulin? Results from a nationwide French survey**

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**Background and aims:** The IDAHO (Insulin initiation in type 2 Diabetic patients At HOspital) French registry was implemented to gather information about the characteristics, management and 1-year outcome of type 2 diabetic patients for whom in-hospital insulin therapy was initiated.
**Materials and methods:** Of the 303 French hospital departments used to routinely initiate insulin, 232 (77%) participated in the study. In each center, all consecutive insulin-naïve type 2 diabetic patients admitted in June 2001 and for whom insulin treatment was initiated during the hospital stay had to be included. The survey was performed before the launch of the long-acting insulin glargine.
**Results:** Among the 797 patients included in the survey, 433 (54%) received basal insulin (281 NPH once daily, 139 NPH bid, 13 insulin zinc od). They were 64 ± 12 years old, 54% were females, and their mean BMI was 29 ± 6Kg/m$^2$; their median diabetes duration was 11 years; 57% had a micro- or macrovascular complication; their mean HbA1 c was 9.9 ± 2.1% and their mean fasting plasma glucose (FPG) was 2.2 ± 0.9g/l; their oral treatment prior to insulin introduction included no oral antidiabetic drug (OAD) in 6% of cases, a single OAD in 20% of cases, 2 OADs in 52% of cases and 3+ OADs in 21% of cases. After basal insulin introduction, 30% of pts did not received any OAD while 22% of patients continued 1 OAD, 39% of pts continued 2 OADs and 10% continued 3+ OADs. During the 1-year follow up, 302 pts (70%) remained on basal insulin, 57 patients had a reinforcement of insulin therapy, 38 patients discontinued insulin, 22 patients were lost to follow up and 14 patients died. We analyzed the relationship between FBG and HbA1 c at endpoint in the subgroup of patients that remained on basal insulin during the 1-year follow-up and had BG values (251 pts): at endpoint, mean HbA1 c was 7.9 ± 1.4% and mean FBG was 1.5 ± 0.4g/l. The ability of FBG to predict adequate blood glucose control (HbA1 c ≤7%) was established by a receiver operating characteristic (ROC) curve; the cut off point of FBG=1.30 g/l was associated with the highest positive predictive value (43%) and led to a sensitivity of 54% and to a specificity of 74%. The 67 patients (27%) with HbA1 c ≤7% had a mean FBG of 1.27 ± 0.26g/l whereas the 184 patients (73%) with HbA1 c >7% had a mean FBG of 1.54 ± 0.46g/l (p=0.0001). In these 2 subgroups, the respective incidence of symptomatic hypoglycemia was 0.80 ± 1.8 vs 0.44 ± 1.5 event/patient/year (p=0.002).

**Conclusion:** In conclusion, in current practice, only one fourth of type 2 diabetic patients treated with basal insulin achieved the HbA1 c goal of 7%. The mean FBG of the whole population was as high as 1.50 g/l although the target FBG should be at least lower than 1.30g/l to expect achieving such a goal. The availability of the long-acting insulin glargine might facilitate a more aggressive titration thanks to its flat action profile leading to a reduced risk of hypoglycemia.

## 319

**The haemoglobin glycation index is reproducible in dysglycaemic individual but is not explained by post-challenge plasma glucose levels**

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**Background and aims:** The haemoglobin glycation index (HGI) measures whether a subject's $HbA_{1c}$ is higher or lower than typical for their fasting plasma glucose (FPG). We used Early Diabetes Intervention Trial (EDIT) data to determine whether HGI values are reproducible and whether differences relate to two-hour post-challenge plasma glucose levels (2HPG).
**Materials and methods:** $HbA_{1c}$ and 75g OGTT data were available at baseline and at three years on 271 of 631 EDIT subjects recruited with two FPG levels 5.5–7.7 mmol/L inclusive two weeks apart. Using 1985 WHO criteria, 100 (36.9%) had normal glucose tolerance (NGT), 22 (8.1%) had isolated impaired fasting glucose (IFG), 69 (25.5%) had isolated impaired glucose tolerance (IGT), 38 (14.0%) had both IFG and IGT and 42 (15.5%) had diabetes. HGI was defined as the 'residual' from a linear regression model of $HbA_{1c}$ on FPG *i.e.* an individual's HGI is their actual $HbA_{1c}$ minus that predicted from their FPG by a linear regression model. Subject's 0 and 3-year HGI values were compared and regression model used to determine whether HGI values are explained by 2HPG, age, sex, race, body mass index, HDL cholesterol, systolic blood pressure, fasting plasma insulin and smoking status.
**Results:** 0 and 3-year HGI values were correlated (Spearman's r=0.61, p<.0001). In a multivariate mode for 3-year HGI values, only 0-year HGI, age and 2HPG were significant at the 5% level. All other variables had p-values > 0.1 except Indian Asian ethnicity (p=0.073). Age and 2HPG together had a $R^2$ statistic of 0.088, indicating that these variables explain less than 9% of the variation in HGI.
**Conclusions:** Dysglycaemic individuals have highly reproducible intra-subject HGI values with inter-subject differences that are not explained by 2HPG levels.

## 320

**The combined effect of cigarette smoking and alcohol consumption on blood sugar level, blood pressure, and serum lipids in male Japanese patients with Type 2 diabetes**

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**Background and aims:** Smoking is associated with premature development of micro- and macro-vascular complications of diabetes and may also have a role in the development of type 2 diabetes. In contrast, light-to-moderate chronic alcohol intake is associated with a decreased risk of coronary heart disease in diabetes. Chronic excessive intake of alcohol is, however, associated with raised blood pressure. To clarify the effect of combined smoking and drinking, we investigated the clinical characteristics of Japanese patients with type 2 diabetes.
**Materials and methods:** We registered 2940 out-patients with type 2 diabetes from April 2000 to May 2001 as part of the hospital-based Shikoku Diabetic Study. Smoking was defined as a current smoker, and non-smoking as a person without any smoking history. Heavy drinking was defined as daily alcohol consumption greater than 30g, light-to-moderate drinking as less than 30g/day, and non-drinking as a person who did not drink alcoholic beverages. The prevalence of both smoking and drinking was higher in males compared to females (smoking: male 39.9% vs. female 7.9%; drinking: male 76% vs. female 14.4%). We therefore analyzed the data of only the male patients and for this purpose divided the patients into six groups. Group 1 (n=357) non-smokers S(−) and non-drinkers D(−); group 2 (n=134) S(−) and light-to-moderate drinkers M(+); group 3 (n=227) S(−) and heavy drinkers H(+); group 4 (n=236) current smokers S(+) and D(−); group 5 (n=102) S(+) and M(+); group 6 (n=330) S(+) and H(+). The mean age of the patients was 58.8 ± 11.1 years, mean duration of diabetes 8.9 ± 7.9 years, mean body mass index 23.9 ± 3.5 kg/m$^2$, mean plasma glucose 169 ± 65 mg/dl, and mean HbA1 c 6.9 ± 1.4%. The mean age of group 1 (61.6 ± 12.4 yr) was significantly higher than group 2 (60.0 ± 10.6 yr), group 3 (59.4 ± 10.2 yr), and group 4 (58.8 ± 10.9 yr), while the mean age of group 4 was significantly higher than group 5 (55.7 ± 10.8 yr) and group 6 (56.1 ± 9.7 yr). Group 3 had a significantly higher mean BMI (24.4 ± 3.1 kg/m$^2$) than either group 1 (23.9 ± 4.0 kg/m$^2$) or group 6 (23.5 ± 3.2 kg/m$^2$).
**Results:** The mean levels of HbA1 c, total serum cholesterol (T-cho), triglyceride (TG), and low density cholesterol (LDL-C) in group 1 at the initial visit were significantly lower than those in group 4 (HbA1 c 6.7 ± 1.3 vs 7.0 ± 1.4%, p=0.013; T-cho 190 ± 33 vs. 196 ± 40 mmHg, p=0.035; TG 141 ± 98 vs 163 ± 109 mg/dl, p=0.011; LDL-C 110 ± 28 vs 118 ± 38 mg/dl, p=0.008). The mean level of high density cholesterol (HDL-C) of group 1 was significantly higher than in group 4 (1 52 ± 13 vs 4 47 ± 14 mg/dl, p=0.000), but was significantly lower than that in group 3 (55 ± 17 mg/dl, p=0.006). Significant differences in mean diastolic blood pressure were observed between groups 1 and 3 (74 ± 11 vs. 77 ± 11 mmHg, p=0.000) and groups 4 and 6 (75 ± 11 vs. 77 ± 10 mmHg, p=0.014).
**Conclusion:** In male Japanese patients with type 2 diabetes, cessation of smoking influences mainly HbA1 c and serum lipids, whereas intake of alcohol influences primarily blood pressure and serum lipid profile.

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**Insulin initiation in Type 2 diabetic patients aged 75+ in the real world**

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**Background and aims:** The IDAHO (Insulin initiation in type 2 Diabetic patients At HOspital) French registry was implemented to gather information about the characteristics, management and 1-year outcome of type 2 diabetic patients for whom in-hospital insulin therapy was initiated.
**Materials and methods:** Of the 303 French hospital departments used to routinely initiate insulin, 232 (77%) participated in the study. In each center, all consecutive insulin-naïve type 2 diabetic patients admitted in June 2001 and for whom insulin treatment was initiated during the hospital stay had to be included.
**Results:** Among the 797 patients included in the survey, 143 were aged 75 and over. The elderly population differed from the younger population by a higher prevalence of hypertension (80 vs 64%) and of macrovascular complications (55% vs. 25%), a lower BMI (27 ± 5 vs 29 ± 6 Kg/m$^2$) but exhibited the same level of FBG (2.5 ± 1.4 vs. 2.3 ± 1.0g/l) and of HbA1 c (10.2 ± 2.4 vs. 10.1 ± 2.2%). The insulin regimen was strongly influenced by age: in the elderly, insulin was often introduced in 2 daily injections (1 injection: 36% vs. 43%; 2 injections: 58% vs. 42%; 3+ injections: 6% vs. 15%; p =0.0008) and the number of associated OADs was strikingly reduced (no OAD: 65% vs. 39%; 1 OAD: 20% vs. 26%; 2+ OADs: 15% vs. 36%; p ≤ 0.0001). In the 670 (elderly :113; youngers : 557) patients that continued insulin at 1 year, mean HbA1 c decreased by 2.1 ± 2.4% and mean fasting blood glucose by 0.75 ± 1.07g/l without difference according to age. The mean final HbA1 c was 7.8 ± 1.6% in the elderly population and 7.9 ± 1.4% in the younger population. The mean target FBG given by the physician to the patient was higher in the elderly (1.46 ± 0.24g/l vs. 1.23 ± 0.19g/l, p ≤ 0.0001) but the mean FBG observed at 1 year was not different (1.47 ± 0.40g/l vs. 1.56 ± 0.49g/l, NS). The occurrence of symptomatic hypoglycemia was similar in the elderly and in the younger population (1.0 ± 3.0 vs 0.9 ± 2.3 event/patient/year, p=0.26). The mean increase in body weight was 2.6 ± 4.9 and 3.4 ± 6.3Kg respectively (NS). Patients'autonomy was evaluated by the percentage of patients ensuring themselves the insulin injections; this percentage was much lower in the elderly population than in the younger population at hospital discharge (38% vs. 80%; p<0.0001) but increased significantly in both subgroups at one year (56% vs. 88%; p<0.0001).
**Conclusion:** This observational survey highlights the specificities of the characteristics and management of elderly type 2 diabetic patients and shows that insulin treatment leads to an acceptable blood glucose control without increasing the risk of hypoglycemia in such population.

# PS 8

# Complications in Type 2 diabetes and the metabolic syndrome

## 322

**Estimating the true impact of diabetes on mortality: evaluation of a model based approach**

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**Background and aims:** It is well known that routine mortality statistics underestimate the number of deaths attributable to diabetes, and that measuring the true impact requires either special studies or modifications to death certification practices that are unlikely in the foreseeable future. The aim of this study was to evaluate an approach, using a generic disease burden model (DISMOD 2) developed for use by the World Health Organization, to estimate diabetes attributable mortality.
**Materials and methods:** Data were used from a population based cohort established in 1994 of 4842 men and women with diabetes and resident in South Tees, UK. Age and sex specific prevalence rates for known diabetes for the total South Tees population were calculated, and the relative risk of dying for people with diabetes vs those without diabetes taken from published studies. Using DISMOD 2 these data were used to estimate both the total number of deaths in the cohort and the deaths attributable to diabetes in the South Tees in one year. The actual number of deaths attributable to diabetes were based on the number of deaths occurring in this cohort in 1995 minus the number of deaths that would have occurred based on the death rates in non-diabetic population (available from routine statistics).
**Results:** The DISMOD 2 approach estimated that 120 men and 95 women from this cohort would have died in 1995, based on underlying mortality rates and the assumed relative risk of death in diabetes. Of these, 38 deaths in men and 37 in women were attributable to diabetes. In reality 130 men and 96 women died in this cohort died in 1995, with 48 deaths in men and 38 deaths in women attributable to diabetes.
**Conclusion:** Further comparisons are needed, but in this example the model based estimates and the actual figures were similar. With realistic assumptions, or data where they exist, on the prevalence of diabetes and relative risk of death the DISMOD 2 software, which is freely available, provides a means of estimating both the total number of deaths in people with diabetes and the deaths attributable to diabetes. Such estimates are essential for a proper consideration of the public health impact of diabetes.

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**Realistic estimates of excess deaths attributable to diabetes**

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**Background and aims:** Mortality due to diabetes is underestimated in statistics based on death certification. The aim of this study was to provide more realistic estimates of the global number of excess deaths attributable to diabetes in the year 2000.
**Materials and methods:** The World Health Organization uses a computerized generic formal disease model (DisMod II) to assess disease burden in the absence of specific data on incidence, prevalence and mortality. This model was used to estimate excess worldwide mortality attributable to diabetes. Input data included age-specific diabetes prevalence from earlier WHO estimates, and relative risk of dying for people with diabetes vs. the non-diabetic population from published studies. The results were validated with independent methods.
**Results:** Excess global mortality attributable to diabetes is estimated at 3.2 million deaths in the year 2000 (0.9 million in developed and 2.3 million in developing countries). This is approximately 1 in 20 deaths. In Europe over 600,000 deaths were attributable to diabetes. In adults 35–64 years old, diabetes accounted for 16–22% of all deaths in Eastern Europe, and around 10% in Western Europe. Globally, 1 in 50 deaths in low-income countries and 1 in 10 deaths in high income countries can be attributed to diabetes.
**Conclusion:** The global number of deaths attributable to diabetes is at least 3 times higher than reported in official reports based on death certification, such as the annual World Health Report. In Europe, diabetes is a substantial cause of premature mortality in adults. Due to lack of data, the relative risk of dying for persons with diabetes 80+ years old was assumed to equal 1, and this could have resulted in the underestimation of the number of excess deaths attributable to diabetes in Europe.

## 324

**Excess winter mortality in diabetic subjects**

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**Introduction:** Seasonal studies show highest all-cause mortality rates in winter. Air temperature and respiratory infections may contribute to this variation.It has ben proposed that lower socioeconomic status may be associated with excess winter mortality. No studies have specifically analysed seasonal mortality in diabetic subjects.
**Aim:** To ascertain the seasonal variations in deaths in diabetic subjects and to explore possible links between causes of death, average air temperatures and socio-economic status.
**Methods:** Seasonal variation of death was studied over 8 years in 1736 subjects. These patients were drawn from the South Tees Diabetes Mortality Study cohort, which comprised 4842 patients (Male 55%; Type 2 diabetes 84%). Causes of death were extracted from death certificates obtained from the Office for National Statistics and coded using ICD-10 rules. Townsend scores were used to assess the relationship between seasonal deaths and socio-economic class. Temperatures from 1994–2002 were obtained from the Meteorological Office (UK) and monthly deaths were correlated to the average monthly temperatures.
**Results:** 36% of the South Tees Diabetes Mortality Study cohort (1736 patients; male: 55%) died between January 1994 and December 2002. All cause mortality was highest in January (Crude mortality rate 4.17%, 95% CI: 3.60–4.75;mean monthly temperature 4° C,range 2.5–5.8) and lowest in July (Crude mortality rate 2.54%, 95% CI: 2.09–2.99; mean monthly temperature 16° C, range 14.5–16.7). Mortality increased in December and peaked in January. Circulatory disease (59%) was the major cause of death with 40% of deaths due to ischaemic heart disease (IHD). Mortality from IHD was highest in January (Mortality rate 1.76%,95% CI: 1.39–2.13) and lowest in July (Mortality rate 0.87%,95% CI: 0.61–1.13). 38% of deaths observed in the cohort occurred during the winter months of December to March. Deaths from respiratory disease also peaked in January. No seasonal variation was observed for deaths due to malignancy and cerebrovascular disease. For IHD,an inverse relationship between deaths and socio-economic status was observed in January but not July. Deaths from IHD in January were 50% higher in the most deprived group compared to the most affluent group.
**Conclusion:** There was excess diabetic mortality in winter with deaths mainly from circulatory disease peaking in January. Winter mortality due to IHD was highest in the poorest socio-economic group who may benefit from warmer and well insulated houses.

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**Risk and outcome of community-acquired bacteraemia with enterobacteria in patients with diabetes mellitus**

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**Background and aims:** Diabetic subjects are generally considered to be at high risk of severe bacterial infections. Gram-negative infections originating from the urinary tract seem to be a common clinical problem in diabetes mellitus. Epidemiological studies of the association between diabetes and bacteraemia caused by gram-negative bacilli such as E. coli and other enterobacteria are limited. We therefore conducted a population-based study to examine whether patients with diabetes have an increased risk of or a poorer prognosis following community-acquired Enterobacteriaceae bacteraemia as compared to that of non-diabetic patients.
**Materials and methods:** The risk analysis included 1317 patients older than 15 years with a first hospitalisation for community-acquired bacteraemia due to the family Enterobacteriaceae ascertained between 1992 and 2001 in the County Bacteraemia Registry. For each case 10 gender- and age-matched population controls were selected. Diabetes was verified by

record-linkage with the County Prescription Database and the Hospital Discharge Registry. The risk of bacteraemia among diabetic subjects and non-diabetic subjects was estimated by conditional logistic regression adjusted for a range of comorbid diseases using Charlson's comorbidity index. An outcome analysis for diabetic and non-diabetic patients with bacteraemia was done using a Cox proportional-hazard regression analysis to adjust for possible confounding factors.
**Results:** 225 cases (17.1%) had diabetes diagnosed before admission as compared to 779 (5.9%) of controls. The adjusted odds ratio (OR) for enterobacterial bacteraemia in patients with diabetes was 2.9 (95% CI: 2.4–3.4). The highest relative risk was noted in diabetic adults below 65 years (OR=5.9, 95% CI: 3.9–9.0). Mortality was higher in diabetic compared with non-diabetic patients with bacteraemia (17.3% vs. 13.4% after 30 days). After adjustment for gender, age, comorbidity and focus of infection the 30-day mortality rate ratio for diabetic patients was 1.4 (95% CI: 1.0–2.0) compared with non-diabetic patients.
**Conclusion:** Diabetes is associated with a markedly increased risk for community-acquired bacteraemia due to Enterobacteriaceae. This severe infection also seems to have a poorer outcome in diabetic subjects.

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**Visual impairment, retinopathy prevalence and mortality in a population-based sample of insulin-treated diabetics: gender differences**
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**Background and aims:** Diabetic women have a greater relative risk of death from cardiovascular disease than diabetic men. Recent population-based studies revealed that in the diabetic population there was a higher proportion of visually impaired females than males, with no overall ethnic differences. U.S. Hispanic women were more likely to be visually impaired from diabetic retinopathy, although gender differences were not statistically significant. Retinopathy is independently associated with cardiovascular mortality. The purpose of this study was to examine the data suggesting that diabetic females have greater low vision prevalence and to try finding the possible reasons.
**Materials and methods:** The data from the National SINADIAB register on insulin-treated diabetes mellitus patients from Donetsk Region of Ukraine (pop. 4 804 500) has been analyzed. The clinical data set mainly corresponded to the St. Vincent declaration Basic Information Sheet. Low vision (LV) (visual acuity < 0,1 in at least one eye) and proliferative retinopathy (PR) prevalence was compared in 4296 men and 7111 women. In LV and PR groups of DM patients under the age of fifty, 2003 year's total and diabetes-related (DR) mortality incidence was compared. DR and total mortality (TM) were taken into consideration according to ICD10. $\chi^2$-test was used to compare categorical variables.

**Results:**
Table 1: LV and PR Prevalence in Population-Based Sample of Insulin-Treated Diabetics

| Groups | Total (patients) | Low Vision (%) | Proliferative Retinopathy (%) |
|---|---|---|---|
| Male | 4296 | 4.3* | 1.88* |
| female | 7111 | 7.72 | 3.18 |
| male (under 50 years old) | 2085 | 2.88* | 3.12* |
| female (under 50 years old) | 2001 | 5.3 | 5.9 |

*p (male/female)<0.001

Table 2: LV and PR Mortality Rates (2003) of Insulin-Treated Diabetics Under the Age of 50

| Groups | Low Vision (patients) | Total Mortality (%) | Diabetes Related Mortality (%) | Proliferative Retinopathy (patients) | Total Mortality (%) | Diabetes Related Mortality (%) |
|---|---|---|---|---|---|---|
| Male | 60 | 16.66* | 11.66 | 65 | 9.23 | 9.23* |
| Female | 106 | 5.66 | 3.77 | 118 | 4.23 | 3.39 |

*p (male/female)<0.05

LV and PR are more common in diabetic women than men (Table 1). In male/female groups under 50 the number of patients for each group was about the same. Therefore, the revealled phenomenon is not linked to a higher age of females. Probably it can be explained by greater male total mortality among the LV patient group, as well as higher DR male mortality in the PR group (Table 2). All DR mortality cases were the end stage of renal failure.
**Conclusion:** Thus, population-based study allowed us to confirm the data on greater prevalence of low vision and proliferative retinopathy in diabetic women.
*Supported by: National Diabetes Mellitus Programme*

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**Prevalence of the metabolic syndrome and its impact on all-cause and cardiovascular mortality in three Asian origin populations**
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**Background and aims:** Few studies have examined the impact of the metabolic syndrome as defined recently by WHO and ATP III on mortality and no studies have analysed this in Asian populations. Thus the aim of this study was to assess the gender-specific age-standardized prevalence of the metabolic syndrome and its impact on all-cause and cardiovascular disease mortality in Asian origin populations.
**Materials and methods:** The study was based on three prospective cohort studies comprising 2523 males and 2915 females aged 30–89 years, with a median follow-up of 5.0 years. The metabolic syndrome was defined according to WHO and ATP III. WHO defines the metabolic syndrome as: glucose intolerance (Impaired Glucose Regulation and/or diabetes) and/or insulin resistance (top quintiles of fasting insulin concentrations in non-diabetes), together with two or more of the following: obesity, hypertension, raised triglyceride, or low HDL cholesterol. ATP III defines the metabolic syndrome as: three or more of the following components: obesity, hypertension, raised triglyceride, low HDL cholesterol or fasting hyperglycemia (FPG over 6.1 mmol/l and/or diabetes). Hazard ratios for all-cause and cardiovascular disease mortality were estimated with Cox models in each cohort and meta-analyses was used to assess the overall association of the metabolic syndrome with mortality risk.
**Results:** No major difference in the age-standardized prevalence of metabolic syndrome was found using the two proposed definitions (all p-values over 0.1). The age-standardized prevalence of the metabolic syndrome varied across three cohorts with raging from 16.7% to 39.0% for males and from 13.5% to 30.5% for females. During follow-up, 319 deaths were recorded (149 from cardiovascular disease). The overall hazard ratios (95% confidence interval) for all-cause and cardiovascular disease mortality in subjects with the WHO metabolic syndrome versus those without it were 1.21 (0.86–1.72) and 1.44 (0.88–2.38) in males and 1.55 (1.01–2.39) and 1.96 (1.05–3.64) in females after adjustment for age, cholesterol, and smoking, respectively. Using the ATP III definition the corresponding hazard ratios were: 1.39 (1.01–1.92), 1.69 (1.05–2.71), 1.51 (1.01–2.28) and 2.38 (1.30–4.33).
**Conclusion:** The WHO and ATP III definition of the metabolic syndrome gave similar prevalence estimates of the metabolic syndrome in all three cohorts, but ATP III identified individuals at higher risk of dying from all causes and from cardiovascular diseases.
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**Prevalence of the metabolic syndrome and associated novel cardiovascular risk factors in patients with Type 2 diabetes**
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**Background and aims:** Conventional risk factors such as plasma lipids and hypertension only partly account for the excess risk of developing cardiovascular disease in type 2 diabetes mellitus (T2D). Recent evidences suggest that conditions associated with T2D, such as insulin resistance (IR), may also play a role in „regulating" cardiovascular risk factors. IR seems to be a an underlying pathogenic factor in the development of the metabolic

syndrome (MS). We assessed the association of MS based on definition of NCEP-ATP III with non-traditional cardiovascular risk factors in patients with T2D.
**Materials and methods:** The sample comprise 1961 patients (age 61.6 ± 7 yrs; 42% women (W), 58% men (M); diabetes duration 12 ± 10 yrs). As detailed in the ATPIII report, participants having 3 or more of the criteria were defined as having the MS. Glycated hemoglobin A1 c (HbA1 c), total, LDL and HDL cholesterol, uric acid, urinary albumin/creatinine ratio (UAlb/Cr), and fibrinogen were measured.
**Results:** The prevalence of the MS in men and women was 73,4% and 96% respectively. The prevalence increased from 18% before 40 years of age to 70% after 80 years. Diabetes duration was not different in patients with MS than those without MS (M: 11.5 ± 10 vs 13 ± 9; F: 11.3 ± 9 vs 9.0 ± 9 yrs). The number of components of the MS was related to age (ANOVA $p<0.05$) but not to diabetes duration. The prevalence of central obesity was 56% in men and 95% in women; hypertension 67% and 74%; low HDL-C 26% and 25% and high triglycerides were 41% and 43%, respectively. The levels of HbA1 c was increased in men with MS (7.7 ± 1.2 vs 7.4 ± 1.2%, $p<0.01$), while uric acid (F: 5.1 ± 1.4 vs 3.5 ± 5.1 mg/dl, $p<0.0001$; M: 5.7 ± 1.4 vs 5.2 ± 2,3 mg/dl, $p<0.001$) and non-HDL-C (F: 162 ± 38 vs 141 ± 34 mg/dl, $p<0.01$; M: 158 ± 39 vs 142 ± 36, $p<0.001$) was higher both in men and women with MS. No difference was observed for LDL-C and fibrinogen concentrations. The rate of microalbuminuria increased significantly in both men and women in thepresence of none (13% vs 1.4%), one (13% vs 2.8%), two (20% vs 38%), three (26% vs 33%) or four (22% vs 24%) components of the MS.
**Conclusion:** These results show that the MS is highly prevalent in type 2 diabetes. The levels of novel cardiovascular risk factors are increased in diabetics with MS and may identify a subgroup at high risk. The development of comprehensive efforts directed at controlling the components (mainly obesity) of MS are urgently needed in type 2 diabetes.

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### Comparison of the prognostic value of the metabolic syndrome and the Framingham risk equation in determining cardiovascular outcomes in newly diagnosed Type 2 diabetes: results of a United Kingdom prospective study

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**Background and aims:** The 2001 National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III definition of metabolic syndrome has been shown in a cross-sectional study to be associated with an increased prevalence of coronary heart disease. We have prospectively investigated the effectiveness of the definition as a clinical tool to assign risk of primary cardiovascular (CVD) and coronary heart disease (CHD) in people with newly diagnosed type 2 diabetes when compared to the prognostic value of the Framingham Risk Equation.
**Materials and methods:** 571 (337 males, 234 females) aged between 30 and 74 years and newly diagnosed with type 2 diabetes between 01/05/1996 and 30/06/1998 were entered in the initial cross-sectional analysis to determine the prevalence of metabolic syndrome and the prevalence of CVD and CHD in those with and without the NCEP ATPIII defined metabolic syndrome. Of these 428 (241 males, 187 females) individuals who were free of manifest cardiovascular disease and remained registered with the Dorset Health Authority till the end of the study on 31/07/01 were entered into the prospective component of the study. Actual primary CVD and CHD events during the study period were recorded. Metabolic syndrome criteria were directly applied besides for waist-hip ratio where a literature-derived body mass index equivalent was used. The sensitivities and specificities of the metabolic syndrome definition and a Framingham-derived 15% 10-year CHD risk threshold (based on current United Kingdom guidelines) in identifying cohort members with primary CVD and CHD events were compared.
**Results:** The prevalence of the metabolic syndrome at diagnosis was 462/557 (82.9%). In males the prevalence was 258/337 (76.6%) and in females, 204/234 (87.1%). The prevalence of CVD at diagnosis of type 2 diabetes was 99/462 in those with the metabolic syndrome and 13/95 in those without (21.4% vs. 13.7% ($p=0.046$)) and for CHD the respective figures were 73/462 and 6/95 respectively (15.8% vs. 6.3% ($p=0.038$)). In the prospective analysis, the sensitivity of the metabolic syndrome was identical to that of the 15% 10-year CHD risk threshold, 0.857 (95% CI 0.782–0.914) and 0.857 (95% CI 0.778–0.915) respectively. The specificity of the metabolic syndrome definition was significantly poorer than that of the multifactorial risk threshold, 0.185 (95% CI 0.163–0.202) and 0.330 (0.307–0.347). However the addition of an age threshold of 50 years and above to the metabolic syndrome criteria improved specificity to 0.421 (0.396–0.442) and demonstrated a similar sensitivity of 0.786 (95% CI 0.70–0.856).
**Conclusion:** We have shown that the metabolic syndrome is present in more than four-fifths of people with newly diagnosed diabetes with a trend towards a greater prevalence in women. The prevalence of CHD is two and a half times greater in those with the metabolic syndrome and diabetes than in those with diabetes alone. In relation to identifying individuals for targeted cardiovascular risk factor reduction, our data shows that the metabolic syndrome definition could be used as a practical alternative to the Framingham-derived 15% 10-year CHD risk threshold, especially in the over-50 age group with newly diagnosed type 2 diabetes.
*Supported by: a research grant from Diabetes UK*

# PS 9
# Prevalence of Type 2 diabetes

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**Prevalence of diabetes mellitus and impaired glucose tolerance in a Canarian population: the Telde study**

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**Background and aims:** Preliminary studies had indicated that the prevalence of Diabetes Mellitus could be unexpectedly high in the Canary Islands.
This study was aimed to estimate the prevalence of type 2 diabetes mellitus, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) in Telde, a Municipality of Gran Canaria assumed to be representative of the whole Canarian Archipelago.
**Materials and methods:** Cross-sectional, population-based survey that included a random sample of 1030 subjects (> 30 years old), stratified by 10-year strata and sex. Each subject underwent a medical questionnaire and physical examination. All, except those with a previous diagnosis of diabetes, performed an oral glucose tolerance test, and 1999 WHO criteria were used for diagnostic classification.
**Results:** The crude global prevalence of diabetes was 12.5% (6% undiagnosed). The age-adjusted prevalence was 13.2% (CI 11.1–15.2), 15.8 (11.8–19.8) in men and 10.6 (7.1–14.1) in women. The adjusted prevalence of IGT and IFG was 11.4% (9.5–13.4) and 2.8% (1.8–3.8), respectively. Standardized for the Segi world population aged 30–64 years, the prevalence of diabetes was 9.9% (8.0–11.9). Age, waist circumference, tryglicerides, hypertension and familial diabetes were independently associated with diabetes (except for hypertension in women). Significant predictors of IFG/IGT in men were age, waist circumference, tryglicerides and low educational level. In women IFG/IGT was associated with age, BMI, tryglicerides, familial diabetes and sedentary lifestyle.
**Conclusion:** The prevalence of diabetes mellitus among the Canarian population exceeds that found in most other Spanish and European communities.
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**Socio-economic difference in prevalence of diabetes in a population living in the same geographical region**

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**Background and aims:** To compare the prevalence of type 2 diabetes (T2DM) and impaired fasting glycemia (IFG) between two different social classes in the same geographical location.
**Materials and methods:** The study was carried out in Dhaka City. Following a multistage cluster sampling, we selected 4 city-wards for slum (poor social class) and 6 adjacent city-wards for non-slum (rich social class) dwellers. Next, we took cluster of families in slum, and households in non-slum city-wards. Then, we investigated 1536 subjects from the poor and 2621 from the rich of age group 20 years or more. We interviewed for occupation, physical activities, family income, education and sanitation. We took height, weight, waist- and hip-circumference. Body mass index (BMI) and waist-to-hip ratios were calculated. Blood pressure was measured. Finally, fasting plasma glucose was estimated. American Diabetes Association diagnostic criteria (1997) were used.
**Results:** The overall prevalence of T2DM was 7.4% in the slum and 13.4%) in the non-slum population and there was significant difference between them (chi-sq: 34.1, $p<0.001$). The prevalence of IFG was also significantly higher in the rich than in the poor (7.1 vs. 4.1%, $p<0.001$). The mean family income was lower and the mean duration of physical activities was higher in the poor than the rich class. Illiteracy rate was also higher among the poor (65 vs 35%). The mean (SD) values for all anthropometric measurements (height, weight, waist, hip, BMI, WHR) were found significantly lower in the poor group than their rich counterparts (for all $p<0.001$). The values (mean and SD) for systolic and diastolic blood pressure were also found to be lower in the poor. With regards to blood pressure, only systolic hypertension was more prevalent among the rich than the poor (8.5 vs. 5.9%, $p<0.01$), whereas diastolic hypertension did not differ. Importantly, the mean (SD) of fasting plasma glucose was higher in the rich than the poor [4.8 (2.4) vs. 4.3 (1.4) mmol/l, $p<0.001$].
**Conclusions:** The prevalence of diabetes in the rich was significantly higher than their poor counterpart living in the same geographical location. The rich people were relatively more obese, more hypertensive and more hyperglycemic, possibly, due to less physical activities, excess mental stress and high calorie intake. This study did not include calorie intake. Further studies are needed to identify the risk factors for increased prevalence of diabetes among the rich social class.
*Supported by: BIRDEM*

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**Increasing prevalence of Type 2 diabetes in the young adult population**

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**Background and aims:** Diabetes clinics in North America have reported increasing numbers of young adults with Type 2 Diabetes (T2DM). Our aim was to characterise type 2 diabetes in young adults attending the diabetes clinics of a university teaching hospital in the United Kingdom.
**Materials and methods:** Using an electronic database, we undertook a retrospective audit to identify people with T2DM aged 16 to 30 years. Data was analysed for demographic variables, features of metabolic syndrome, glycaemic control and complications.
**Results:** Forty patients were identified, 25 female and 15 male, with mean age 25.45 years and mean duration of diabetes 3.46 years. The number of T2DM in young adults diagnosed per year rose from 1 in 1991 to 4 in 1997, and to 10 in 2003.
Their glycaemic control is satisfactory, mean HbA1 c (mHbA1 c) being 7.87% (± 2.26%) and 42.5% having mHbA1 c <7.0%. Fourteen had hypertension (35%), retinopathy was present in 2 and microalbuminuria in none of the patients. Nine patients (22.5%) also had an elevated serum alanine aminotransferase (ALT). Twenty-one patients (52.5%) were on oral hypoglycaemic agents alone, 6 (15%) on combination of Metformin and insulin, 10 (25%) on insulin only and 3 (7.5%) on dietary modification alone.
90.6% had body mass index (BMI) >24.9 kg/m$^2$, and 75% had a BMI >29.9 kg/m$^2$. 50% had total cholesterol (TC) >5.0 mmol/l, 52.5% had triglyceride (TG) >1.7 mmol/l and 15.2% had high-density-lipoprotein cholesterol (HDL) <0.9 mmol/l. 42.5% had a family history of T2DM (FH), 24% of the female patients had polycystic ovarian syndrome (PCOS) and acanthosis nigricans (AN) was identified in 15% of patients.
Fourteen patients (35%) had South Asian descent (SA) with mHbA1 c 8.16% (± 1.74%) compared to 7.70% (± 2.51%) in the other patients ($p=0.54$). All SA patients had a BMI >24.9 kg/m$^2$ and 77.8% had a BMI >29.9 kg/m$^2$. 57.1% of patients had TC >5.0 mmol/l and 50% had TG >1.7 mmol/l, but none had HDL <0.9 mmol/l. FH was positive in 64.3%, 21.4% females had PCOS while AN was identified in 35.7% of patients.
**Conclusion:** This increasing population of young adults with T2DM do not have a particularly poor glycaemic control, but do have other features of metabolic syndrome. Early lifestyle interventions are crucial at this stage, and aggressive treatment of features of the metabolic syndrome will be required to prevent cardiovascular disease.

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**Ethnicity and gender are strong predictors for diabetes in an urban western society – implications for prevention**

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**Background and aims:** Ethnic origin may indicate different susceptibilities for disease due to genetic factors or different exposure for environmental risk factors. The aim of this study was to identify subgroups at special risk of developing type 2 diabetes in an urban western society.
**Materials and methods:** We performed a population based cross-sectional survey of 31–67 year olds in a multi-ethnic district in Oslo, collecting data from questionnaires, physical examination and serum analyses. Subjects with non-fasting serum glucose (NFSG) ≥ 6.1 mmol/l were asked to return for a fasting sample. The prevalence of known diabetes was based on self-reported data. Undiagnosed diabetes was estimated from blood samples. Among 6123 invited, 2933 subjects (48%) attended, of whom 21.4% were immigrants.

**Results:** The age adjusted diabetes prevalence for the 30–67 year olds was 7.2% (95% CI: 5.6–8.8) in western men and 3.3% (2.3–4.3) in women. Few immigrants were >60 years. For the 30–59 year olds the prevalence was 27.5% (18.1–36.9) and 14.3% (8.0–20.7) in South Asian women and men versus 2.9% (1.9–3.4) and 5.9% (4.2–7.5) in western. The impact on diabetes prevalence of age, body height, weight at 25 years and income, differed significantly between western and South Asian men and women. Income and education were strongly associated with diabetes in western subjects. For South Asian compared to Western women the age adjusted OR (95% CI) for diabetes was 11.0 (5.8–21.1), and for men 3.0 (1.6–5.4), and 7.7 (3.9–15.3) and 2.6 (1.4–4.9) when adjusted also for waist to hip ratio (WHR). OR for ethnicity was significantly different from 1.0 even after adjustment for education or income for both genders. In the fully adjusted model (age, WHR, income and body height) the OR for ethnicity was 2.8 (1.3–6.2) for women, but non-significant for men. Non-western immigrants were more physically inactive, and had a higher preference for soft drinks with sugar and daily us of full fat milk than the western population.
**Conclusion:** The age adjusted prevalence of diabetes and several risk factors were significantly higher among subjects from the Indian subcontinent, than in the western population. After adjustment for anthropomteric, behavioural and SES factors there was still an unexplained ethnic difference in women. The most susceptible gender differed between the ethnic groups, being men among the western and women among subject from the Indian subcontinent. This highlights the importance of environmental factors even if genetic susceptibility may be of importance at both the individual and the ethnic level. When it comes to preventive strategies to reduce the huge burden of diabetes among immigrants, the results indicate the need for culture sensitive interventions.

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### Prevalence of undiagnosed pathological glucose metabolism in patients undergoing elective coronary angiography

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**Background and aims:** Patients with acute myocardial infarction show a high prevalence of previously undiagnosed type-II diabetes (DM) or impaired glucose tolerance (IGT). Since there are no data on the true prevalence of IGT or DM in patients with symptoms of stable coronary artery disease (CAD) available, our aim was to verify glycometabolic state of patients undergoing elective coronary angiography for suspected CAD.
**Materials and methods:** We enrolled 160 consecutive patients fulfilling the criteria for elective coronary angiography and all of them, except those with known diabetes, underwent an oral glucose tolerance test (oGTT) with 75g glucose. Frequencies were compared by the Chi-square-test. Relative risk (RR) and 95% confidence intervals (95% CI) were calculated. The criterion for statistical significance was $p<0.05$. Receiver-operating-characteristic (ROC) analysis was done for fasting blood glucose and HbA1 c.

**Results:**

| | DM known | DM new | IGT | NGT |
|---|---|---|---|---|
| n | 51 | 31 | 33 | 45 |
| (%, 95% CI) | (32, 23–42) | (19, 12–28) | (21, 13–30) | (28, 19–38) |
| CAD (n) | 42 | 24 | 24 | 23 |
| (RR, 95% CI) | **(1.61, 1.16–2.14)** | **(1.47, 1.06–2.05)** | | (Reference) |
| multi-vessel CAD (n) | 35 | 14 | 18 | 16 |
| (RR, 95% CI) | **(1.93, 1.23–3.06)** | (1.41, 0.87–2.38) | | (Reference) |
| PTCA ± stent (n) | 19 | 11 | 10 | 9 |
| (RR, 95% CI) | (1.86, 0.90–4.10) | (1.64, 0.80–3.61) | | (Reference) |
| CABG scheduled (n) | 8 | 3 | 5 | 4 |
| (RR, 95% CI) | (1.77, 0.52–6.73) | (1.41, 0.41–5.38) | | (Reference) |
| Total revascularisation (n) | 27 | 14 | 15 | 13 |
| (RR, 95% CI) | **(1.83, 1.06–3.31)** | (1.57, 0.90–2.88) | | (Reference) |

NGT: normal glucose tolerance, Total revascularisation means PTCA±stent or scheduled CABG. Of the 109 patients, without previously known diabetes, 65 (59%) had fasting blood glucose below the threshold for impaired fasting glycaemia of 5.6 mmol/L. ROC-analysis identified fasting blood glucose as a possible predictor for new DM (AUC = 0.66, p=0.01) and HbA1 c for new DM (AUC = 0.7, p=0.002) as well as for new DM & IGT (AUC = 0.71, $p<0.001$).
**Conclusion:** Patients undergoing elective CA show a high prevalence of undiagnosed DM or IGT. Only 28% have normal glucose tolerance. The degree of CAD is positively associated with the presence of pathological glucose metabolism. Therefore we suggest, that an oGTT is absolutely necessary to find out the true glycometabolic state in patients undergoing elective coronary angiography. The detection of pathological glucometabolism is important for further risk factor management in every patient and for the prevention of progression from IGT to manifest diabetes.

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### Gender aspects on interaction between family history of diabetes and lifestyle factors in Type 2 diabetes; a study in middle-aged Swedish men and women

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**Background and aims:** Type 2 diabetes is considered a disorder caused by both genetic and environmental factors. Thus, interplay between a diabetic heredity and lifestyle related components might contribute in the development of type 2 diabetes. With a gender specific perspective, we investigated the associations between family history of diabetes, alone or in combination with other risk factors, and type 2 diabetes. Risk factors assessed were BMI, physical inactivity, smoking, and a psychosocial factor, sense of coherence (SOC).
**Materials and methods:** This population-based cross-sectional study comprised Swedish individuals, 3128 men and 4821 women, aged 35–56 years, without previously diagnosed type 2 diabetes, living in the Stockholm area. Half of the participants had a family history of diabetes, FHD+ (at least one 1st ° relative or at least two 2nd ° relatives with diabetes), and half were without family history of diabetes, FHD-. An oral glucose tolerance test identified, according to WHO criteria 1999, 65 men and 63 women with type 2 diabetes. Information on life-style factors was obtained from a questionnaire. Prevalence odds ratios (POR), accompanied by 95% confidence interval (CI) and controlled for confounders, were estimated in multiple logistic regression analysis.
**Results:** POR for diabetes associated with FHD+ was higher in men then in women, POR=3.1 (CI: 1.7–5.6) and POR=1.7 (CI: 1.0–3.0), respectively. In women, compared to normal weight (BMI<25) with FHD-, the effect of being overweight (BMI≥25) with FHD- increased POR significantly and to higher values than the effect of being normal weight with FHD+, POR=4.3 (CI: 1.4–13.1) vs POR=1.5 (CI: 0.4–5.3). In contrast, in men both ratios were equally high, POR=10.7 (CI: 1.4–81.9) vs 12.4 (CI: 1.6–96.2). The joint effect of being overweight and FHD+ showed the strongest increase, POR for diabetes was 7.9 (CI: 2.8–22.2) in women and 27.3 (CI: 3.7–200.0) in men. Being physically inactive during leisure time in combination with FHD- increased the risk of having diabetes, POR= 2.8 (CI: 1.0–8.2) in women and POR=3.9 (CI: 1.2–12.6) in men, compared to physically active with FHD-. Notably, the same magnitude of POR was reached in physically inactive women with FHD+, POR=3.1 (CI: 1.3–7.2), while the risk was further increased in physically inactive men with FHD+, POR=9.5 (CI: 4.1–22.1). Compared to non-smoking with FHD-, current smoking with FHD- was not significantly associated with diabetes in either gender, whereas current smoking in combination with FHD+ increased the risk two-fold in women, POR=2.0 (CI: 0.9–4.6) and four-fold in men, POR=4.4 (CI: 2.0–10.1). There was no association to diabetes for low SOC combined with FHD-. In the presence of FHD+ the risk of having diabetes was increased, although to similar values within gender irrespective of level of the psychosocial factor; POR=2.0 (CI: 1.0–4.0) for high and POR=2.6 (CI: 1.1–6.1) for low SOC in women, and POR=3.5 (CI: 1.7–7.1) for high and POR=3.9 (CI: 1.5–9.8) for low SOC in men.
**Conclusion:** Our data indicate that the impact of family history of diabetes on the risk of type 2 diabetes diagnosed at middleage was greater in men than in women. Lifestyle related factors, such as BMI and physical inactivity, seem however more important in women.

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### The impact of HIV/AIDS on diabetes prevalence and diabetes healthcare needs in South Africa- projections for 2010

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**Background and aims:** Sub-Saharan African countries have multiple disease burdens, with conditions relating to poverty and underdevelopment coexisting with emerging chronic diseases, injuries and HIV/AIDS. Chronic diseases like diabetes are receiving a low priority, partly due to the misconception that the adult population will be decimated by HIV/AIDS

and few will live long enough to develop diseases such as diabetes and hypertension. We examined the impact of HIV/AIDS on the projected total number of people with diabetes in South Africa in 2010.
**Materials and methods:** Estimates were calculated using recent national census data (2001), current estimates of the burden of HIV/AIDS, age-specific, population-specific and rural and urban-specific diabetes prevalence rates from epidemiological studies (1991–1997) and with two projected changes in prevalence rates (50% and 100% increases). Population projections are based on a AIDS and demographic model that has been calibrated to the ante-natal prevalence, the reported deaths and the population census. In addition, the projected number of previously diagnosed cases were calculated assuming that half of the cases would not be diagnosed.

**Results:** As seen in the table, despite an extensive AIDS epidemic in South Africa, the number of diabetics can be expected to grow.
Demographic impact of HIV/AIDS on projected prevalence of type 2 diabetes and patient load in South Africa

| | 1995 | 2010 Without HIV/AIDS | With HIV/AIDS |
|---|---|---|---|
| Population ('000s) | 40 410 | 52 706 | 47 392 |
| Growth rate (%) | | 1.8% | 1.1% |
| **50% increase in prevalence** | | | |
| Adult Prevalence (%) | 3.6% | 6.2% | 6.2% |
| Projected number of cases ('000s) | 959 | 2 292 | 2 015 |
| Projected patient load ('000s) | 479 | 1 146 | 1 008 |
| **100% increase in prevalence** | | | |
| Adult Prevalence (%) | 3.6% | 8.2% | 8.1% |
| Projected number of cases ('000s) | 959 | 3 027 | 2 631 |
| Projected patient load ('000s) | 479 | 1 514 | 1 316 |

**Conclusion:** Thus South Africa, in common with other sub Saharan African countries, should not lose sight of the impact and importance of the chronic non communicable diseases such as diabetes, in the face of the HIV/AIDS epidemic. Increased resource allocation is critical to improve current low levels of quality diabetes health care. Diabetes primary prevention strategies on a national level require attention.

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**Modelling of Type 2 diabetes mellitus to support causal intervention**
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**Background and aims:** Worldwide nearly 150 million people are suffering from diabetes mellitus; approximately 90 to 95% of them, i.e. 135 to 142 million are type 2 diabetic patients. The development of type 2 diabetes is a discontinuous process, featuring interactions between insulin resistance and insulin secretion. In a given case, it has – until now – been impossible neither to predict nor to establish retrospectively whether one of these latter items might have been the primary event or be dominant in the progression of type 2 diabetes. Therefore, the aim of this study is to assess in a given person the developmental stage along the pathway leading towards type 2 diabetes and to quantify the risk of worsening. The underlying rationale is based upon the hypothesis that the pathogenesis of type 2 diabetes can be modelled mathematically and simulated and thereby be predicted for purposeful intervention to a certain extent.
**Materials and methods:** The modelling procedure was mainly focussed on the construction of the model of pathogenesis of type 2 diabetes, which can be individually identified. Glucose tolerance which is judged by measuring blood glucose concentration after overnight fasting, during daily profiles, or in response to glucose loads provides the basis for definition of developmental stage on the pathway leading to type 2 diabetes. To identify the model in relation to the current level of progression in developing type 2 diabetes in a given person at risk, the individual metabolic situation was analysed by means (i) of simultaneous assessment of glucose tolerance in relation to insulin sensitivity, (ii) of overall endogenous glucose balance, and (iii) of availability of insulin, regardless of whether it is endogenously secreted or exogenously substituted. The figures of these three variables and their alterations during follow-up may allow a prognostic estimate related to specific interventional recommendations.
**Results:** The modelling approach results in a new working version of the well established KADIS® model allowing the identification of individuals in terms of insulin sensitivity and insulin secretion. Moreover, their positioning on the time scale between normal glucose tolerance and the various stages of clinical type 2 diabetes has been rendered possible. This model was verified by means of a clinical-experimental pilot study, where normal persons, people exhibiting different degrees of glucose intolerance + obesity up to type 2 diabetes have been subjected to the model-related identification applying self control data in combination with continuously monitored glucose profiles. Reproducibility has been proven by a clinical follow-up.
**Conclusion:** Based on this model of pathogenesis of type 2 diabetes, the prediction of probability of further development in a particular person provides the chance to intervene more purposefully, e. g. either into insulin resistance or into defective insulin secretion.

# PS 10
# MHC in Type 1 diabetes

## 338

**Contribution of HLA-DR and -DQ haplotypes to clinical heterogeneity in Japanese Type 1 diabetic patients**

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**Background and aims:** Type 1 diabetes mellitus (T1DM) develops both in adult and childhood. Adult-onset T1DM pursue diverse clinical course after its onset. However, it is not clear these clinical phenotype, LADA, slowly progressive Type 1 diabetes, or fulminant Type 1 diabetes represents a late or rapid manifestation of T1DM otherwise distinct clinical entities. To clarify heterogeneity of Japanese adult onset T1DM, we analyzed HLA-DR, DQ haplotype depending on the clinical phenotype and compared them with those of childhood-onset T1DM (CO).
**Materials and methods:** Total of 4,980 adult-onset (>20 years) diabetic patients who registered in hospital based Ehime study was examined in their insulin secretion. Adult-onset TIDM patients were divided by the mode of onset. Acute onset (AO), insulin treatment were initiated < 3 months; intermediate onset, insulin treatment were initiated from 3 to 12 months; slowly onset, insulin treatment were initiated > 12 months after either the diagnosis of diabetes or development of hyperglycemic symptom. In the slowly onset group the patients with GAD antibody was diagnosed as slowly progressive T1DM (SO) and fulminant Type 1 diabetes were diagnosed as reported. Total of 80 CO patients (<18years old) were recruited from the Ehime prefecture. All these CO patients were categorized as acute onset. 190 subjects with a normal glucose tolerance served as control. In the adult-onset T1DM, 19 patients (11 acute onset and 8 SO) were newly recruited to the previously registered T1DM patients in Ehime study.
**Results:** Of the 4,980 patients with adult-onset diabetes, 113 patients (2.3%) were diagnosed as T1DM. In these 113 T1DM patients, 104 could be classified by the mode of onset. Fifty-two patients (50%) were AO, 18 patients (17.3%) were intermediate, 25 patients (24%) were slowly onset of whom 20 patients with GAD antibody were SO, and 9 patients (8.7%) were fulminant T1DM. Two major T1DM susceptible HLA haplotypes in Japanese, *DRB1*0405-DQB1*0401(DR4)* and *DRB1*0901-DQB1*0303(DR9)* significantly increased in AO (P<0.0001) and CO (P<0.0001 and P<0.01), but only *DRB1*0901* allele in SO (P<0.04). AO had a higher frequency of *DR9* than CO. Accordingly, the *DR9/DR4* ratio increased with respect to the age of the onset in acute onset T1DM. Another T1DM susceptible haplotype *DRB1*0802-DQB1*0302(DR8)*, was involved only in CO. Analysis of haplotype combination revealed that *DR4* and *DR9* had dosage effect on AO and CO (P<0.0001), but only *DR9* in SO (P<0.001). *DR8* haplotype showed dosage effect only on CO (P<0.0001).
**Conclusion:** The present study revealed the clinical heterogeneity of adult onset T1DM, more than half of whom are acute onset. *DR9* confers the susceptibility to late onset or slowly progressive TIDM whereas *DR4* shows involvement in early onset TIDM. These results suggest that HLA class II haplotype is an important factor to determine the onset age of TIDM.

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**The impact of HLA haplotype sharing in Finnish families with Type 1 diabetes**

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**Background and aims:** Since 1973 it is known that the major genetic susceptibility to T1DM is coded by genes in the HLA region (6p21.3). It contains more than 200 genes of which many are related to immunity. T1DM is an HLA-associated disease which leads to an immune-mediated destruction of the insulin-producing pancreatic beta cells. Over the last 20 years we have carried out population-based family studies in T1DM in Finland, the country with the highest incidence of T1DM in the world (50/100 000 per year under the age of 15 years).
**Materials and Methods:** Since September 1986 we ascertained nationwide T1DM families through the Finnish Diabetes Registry. All family members (n=6,507) in 1,582 families were fully HLA genotyped. HLA-A,C,B (class I) and HLA-DRB1,DQB1,DPB1 (class II) genotyping was done using PCR-SSP (Dynal kits®) and a sequence-based typing (Visible Genetics®). HLA-A,C,B,DRB1,DQB1,DPB1 haplotypes were defined through segregation.
For this analysis we selected 147 families (9.3%) with two or more siblings with T1DM diagnosed under the age of 15 years (n=147). The average number of children in these families was 2.1. Recombinations were found in two affected siblings (shared 0.5 and 1.5 haplotypes with the proband). These were excluded. We also excluded 7 concordant and 6 discordant monozygotic twins as they are the result of a single meiosis and by definition HLA identical.
**Results:** 66 (56%) affected siblings were HLA identical with the proband (shared 2 haplotypes), 39 (33%) were haplo-identical (shared 1 haplotype) and 14 (11%) non-identical (shared 0 haplotype) compared to the expected 25:50:25% (Table). In 15 siblings HLA identity (shared 2 or 1 or 0 haplotypes) or haplo-identity (shared 1 or 0 haplotype) could not be unequivocally established as one of their parents was homozygous for the whole HLA haplotype (from HLA-A to DPB1). Therefore, we had to devise a new method using a range of maximum and minimum haplotype sharing.
**Conclusion:** Our findings confirm that the major T1DM genetic susceptibility genes are HLA linked. HLA identical siblings have an approximately 100 time greater risk of developing the disease than that in the general population. This has considerable importance for genetic counseling. Also haplo-identical sibling carry a higher risk dependent on whether they share the maternal or the paternal haplotype. Whole HLA haplotypes are more powerful than SNP's or microsatellites.

Table. Haplotype sharing

| Haplotype sharing | 2 | | 1 | | 0 | | Number of subjects |
|---|---|---|---|---|---|---|---|
| Maximum sharing | 76 | 58% | 42 | 32% | 14 | 10% | 132 |
| Minimum sharing | 66 | 50% | 46 | 35% | 20 | 15% | 132 |

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**X-chromosomal linkage study of Finnish Type 1 diabetes families**

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**Background and aims:** *HLA*-region is the major locus (*IDDM1*) in type 1 diabetes (T1D) susceptibility. *HLA*-region explains approximately 50% of the genetic background of T1D suggesting for additional genetic determinants. Several candidate genes and regions have been assigned to T1D. Incidence of T1D is higher in boys than in girls suggesting a possible role for a X-chromosomal locus affecting T1D susceptibility. The strongest evidence for linkage on X-chromosome has been reported at the Xp11-region, but no candidate gene has been identified. We have tested chromosome X for linkage in the Finnish population.
**Materials and methods:** 121 Finnish T1D sibpairs were genotyped with 29 microsatellite markers spanning the chromosome X in ~5 cM intervals. Maximum LOD scores (MLS) were calculated using MAPMAKER/SIBS. Markers were genotyped using fluorescently labelled PCR-primers and automated sequencer (MegaBace 1000). Families were stratified according to four criteria in an attempt to decrease the genetic heterogeneity in the family set; HLA-DQB1-genotypes and IBS sharing, *MspI* -2221 genotypes at IDDM2 and mitochondrial DNA haplogroup sharing. Results: No LOD scores exceeding nominal linkage were detected in the analysis of full data set. Above nominal linkage was detected in four different strata, mtDNA haplogroups H and U, *MspI* -2221 C-allele and sibs sharing 1 or 0 alleles IBS at HLA-DQB1. For mtDNA haplogroup H, LOD scores of 2.2, 1.7 and 1.8 were detected respectively for markers DXS8092, DXS6800 and DXS8076, which span consecutively 8.5 cM. For mtDNA haplogroup U, a LOD score of 1.4 was seen with marker DXS8039. For *MspI* -2221 C-allele, LOD scores of 1.5 and 1.8 was detected with markers DXS1223 and DXS6799 respectively. For IBS1,0 sibs, a LOD score of 1.4 was detected for the marker DXS6810.
**Conclusions:** We found nominal evidence for linkage at the Xp11-region in sibs sharing one or zero alleles at the HLA-DQB1 gene. This result is in

concord with previous report of significant linkage in DR3/DRX sibs, but the effect of the alleged underlying locus to the T1D susceptibility is clearly too weak to be detected and confirmed with the power of the family set used in this study. The linkage detected with sibs sharing predisposing allele C at IDDM2 could suggest an interaction between the two loci. Additionally, markers DXS1223 and DXS6799 are located only few centimorgans from the previously reported suggestive rheumatoid arthritis and autoimmune thyroid disease loci suggesting the existence of common autoimmune susceptibility loci at these regions. Sibs sharing the mtDNA H showed the strongest LOD scores among the strata. Also the fact that the LOD scores were among the highest detected in this study in three neighboring markers gives more credence to the linkage finding and to the potential of mtDNA haplogroups as a method of identifying genetic heterogeneity among data sets. In conclusion, as expected given the size of the family set available, we could not unambiguously confirm any previous X-chromosomal findings or find new susceptibility regions for T1D if $\lambda_s < 1.4$ is assumed for T1D susceptibility loci outside the HLA-region.

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**Different HLA pattern in patients affected by LADA in Congo, central Africa**

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**Background and aims:** Latent Autoimmune Diabetes in Adults (LADA) is characterized by an onset in adult age, presence of GAD antibodies and rapid progression to insulin dependence. The aim of this study was to evaluate the prevalence of antibodies to glutamic acid decarboxylase (GAD) and to tyrosine phosphatase (IA2), and the HLA pattern in an urban population of LADA patients in Congo, central Africa.
**Patients and methods:** We studied 158 consecutive patients, median age 59, affected by non-insulin requiring diabetes diagnosed in adult age and attending the Monkole Centre Hospital in Kinshasa, Congo. As a control group we studied 49 subjects without known family history of diabetes. GAD and IA2 antibodies were measured by IDS recognized radioimmunoessay.
**Results:** HLA-DQB1 typing was detected in 11% of patients, to IA2 in 10%, to both GAD and IA2 in 11%. No significant differences were observed in the high (0201/0302) or moderate (0201/0201; 0302/0302/; 0302/X; 0201/X; X other than 0201, 0302, 0602) risk genotypes between antibody positive, antibody negative patients and controls (0.2% vs. 0.8% vs. 0.3%). Compared with Caucasian LADA patients as reported in the UKPDS an other studies, a striking difference is observed in high and moderate risk genotypes among Africans (1%) vs. Caucasian (12%) LADA patients.
**Conclusion:** These results indicate that the presence of autoantibodies to GAD and IA2 in diabetic patients from central Africa is not associated with the HLA genotypes typical of type 1 diabetes or LADA observed in Caucasian patients. This unique and new finding suggest a different autoimmune process towards beta cell in the African population.

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**HLA-DQB1 alleles and genotypes, islet antibodies and β-cell function in young adult diabetic patients**

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**Background and aims:** Albeit it is well known that HLA is associated with type 1 diabetes, it is unclear how HLA contributes to the pathogenesis of this disease. The aim of the current study was to relate HLA-DQB1 loci to islet antibodies and β-cell function in 1789 recently diagnosed young adults with diabetes.
**Materials and methods:** During a five-year period, 1963 young adults 15–34 years of age were included in the Diabetes Incidence Study in Sweden (DISS). At diagnosis, blood samples were taken for assessment of islet antibodies (ICA, GADA, IA-2A), plasma C-peptide (<0.25 nmol/l low and < 0.10 nmol/l un-measurable), and HLA-DQB1 typing.
**Results:** Islet antibodies were present in 1199 (67%) of 1789 patients. HLA-DQB1 *02, *0302, and *0604 alleles were significantly more frequent in Ab+ vs. Ab- patients (24% vs. 17%, $p < 0.0001$; 35% vs. 16%, $p < 0.0001$; 7% vs. 4%, $p = 0.0018$, respectively). Comparing Ab+ vs. Ab- patients, GADA were closely associated with *02 ($p < 0.0001$), *0302 ($p < 0.0001$) and *0604 ($p = 0.0035$) alleles, whereas IA-2A were closely associated with *0302 ($p < 0.0001$) and *0604 ($p = 0.0008$) alleles. Compared to patients with normal C-peptide levels, patients with low C-peptide levels had significantly higher frequencies of *02 (24% vs. 21%; $p = 0.04$), *0302 (34% vs. 26%; $p < 0.0001$) and *0604 (7% vs. 5%; $p = 0.01$) alleles, whereas the frequency of *0302 allele (32% vs. 26%; $p = 0.023$) was significantly increased in patients with un-measurable plasma C-peptide. HLA-DQB1 *02/0302, *0302/0604 and *0302/X (X = either homozygous allele or any allele other than *02, *0302, *0602-03–04) were significantly more frequent in Ab+ vs. Ab- patients (27% vs. 8%, $p < 0.0001$; 7% vs. 2%, $p < 0.0001$; 26% vs. 12%, $p < 0.0001$, respectively). Compared to patients with normal C-peptide levels, patients with low C-peptide levels had significantly higher frequencies of *02/0302 (24% vs. 18%; p= 0.0027), *0302/0604 (7% vs. 4%; $p = 0.0085$), and *0302/X (26% vs. 18%; p= 0.0008) genotypes, whereas patients with un-measurable plasma C-peptide had increased frequency of the *0302/X genotype (26% vs. 18%; $p = 0.019$).
**Conclusion:** Islet antibodies were associated with HLA-DQB1 *02, *0302 and *0604 alleles; GADA with *02, *0302 and *0604, IA-2A with *0302 and *0604. HLA-DQB1 genotypes comprising the *0302 allele were associated with IA-2A and severe β-cell destruction at diagnosis of diabetes.

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**Type 1 diabetes-protective gene/s map/s in an interval of 4 Mb on rat chromosome 6q32**

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**Background and aims:** Using diabetic BB rats and spontaneously hypertensive rats (SHR), a diabetogenic gene (*Iddm4*) was mapped on chromosome 6q32 showing strong synteny with human chromosome 14q21-32. In this region, diabetes susceptibility loci *IDDM11* and *IDDM16* were identified on 14q24.3-q31 and 14q32.3 by demonstration of significant linkage to microsatellites *D14S67* and *D14S542*, respectively. The strong synteny prompted us to study the importance of *Iddm4* by generating a congenic BB.SHR rat strain recombining a segment of the SHR chromosome 6 into the BB/OK background by serial backcrossing and marker-aided selection, briefly termed BB.6S. The characterization of BB.6S rats demonstrated a drastic reduction of diabetes frequency from 86% to 14% ($p<0.0001$) and a significantly later age at onset of diabetes ($103 \pm 30$ vs. $137 \pm 14$ days; $p<0.001$) in comparison to the parental strain BB/OK. To increase the chance of identification of the appropriate gene(s), subcongenic BB.6S lines were generated and the expression of selected genes was studied.
**Materials and methods:** BB.6S rats were mated with BB, resulting rats were intercrossed, and appropriate homozygotes were selected and inbred. BB.6S (n=43) and their subcongenics (n=154) were observed for diabetes occurrence up to an age of 30 weeks. In addition, the expression of 7 genes located in the region of interest was cross-sectionally studied in blood and spleen of non-diabetic BB, BB.6S and SHR males and females at an age of 30, 70 and 90 days using RT-PCR (ABIPrism7000).
**Results:** One of four newly established subcongenic BB.6S lines showed a diabetes frequency comparable with those of BB.6S (7 diabetics out of 43 vs. 10 out of 49, p=0.79). With the aid of all subcongenic lines, the diabetes-protective region was narrowed down to about 4 Mb located between 133 to 137 Mb on rat chromosome 6q32. In this region, *Yy1*, *WD40*, *Pref-1*, *Rtl1*, *Cdc42*, *Traf3*, and *Tnfaip2* are located. Except *Rtl1*, which was only expressed in spleen, all other genes studied were expressed in both blood and spleen. There were 4 out of 7 genes which showed a comparable behaviour in gene expression in males and in females. The relative expression of *Yy1*, *Pref-1*, and *Cdc42* showed significantly reduced expression in blood of BB.6S and SHR compared with BB/OK at an age of 90 days. In spleen, significantly decreased expression was seen for *Pref-1* at an age of 70 and 90 days and for *RTl1* at an age of 90 days compared with BB/OK rats. Interestingly, the *Rtl1* expression was zero in SHR and was 80% lower in BB.6S rats than in BB/OK at an age of 90 days, also leading to a significant difference between BB.6S and SHR females. A similar behaviour of the gene expression in spleen was also seen for *Yy1* at an age of 90 days, but without significance. All other genes were differently expressed according to gender and strain.
**Conclusions:** Because *Pref-1* is abolished in most tissues after birth, it was shown for the first time that *Pref-1* is expressed in spleen and blood later in life. Interindividual variations in *Yy1* expression of BB rats in blood compared to BB.6S and SHR may be of diagnostic value. In addition, the strong synteny between chromosome 6 and human chromosome 14 suggests, therefore, that the use of genetically and phenotypically well-characterized animal models offers a valuable shortcut to define genetic associations in human diseases in general and in type 1 diabetes in particular.
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**Variation of peripheral CD3⁺ T-lymphocyte content in the diabetic LEW.1AR1-*iddm* rat is associated with a diabetes susceptibility region on RNO1**

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**Background and aims:** The LEW.1AR1-*iddm* rat is an animal model of type 1 diabetes mellitus (T1DM), which arose through a spontaneous mutation within the MHC-congenic inbred strain LEW.1AR1 ($RT1^{r2}$) at Hannover Medical School. FACS analyses of diabetic LEW.1AR1-*iddm* rats and (LEW.1AR1-*iddm* × BN) × LEW.1AR1-*iddm* rats showed variant amounts of blood CD3⁺ T-lymphocytes in the range between 27% and 67%. It was the aim of this study to identify the genetic loci responsible for the phenotype of variant CD3⁺ T-lymphocyte expression and a possible linkage to known diabetes susceptibility loci.
**Materials and methods:** 57 animals of the (LEW.1AR1-*iddm* × BN) × LEW.1AR1-*iddm* backcross population with extreme variations of the CD3⁺ l-lymphocyte content (>30%–< 40% or > 60%) were chosen for linkage analysis using 91 microsatellite marker.
**Results:** 27 animals showed less than 40% of CD3⁺ T-lymphocytes and 30 animals showed more than 60% of CD3⁺ T-lymphocytes. Longitudinal studies indicated that CD3⁺ T-lymphocytes did not fluctuate during the lifespan and were apparently genetically determined. None of the animals exhibited lymphopenia. Importantly the LEW.1AR background strain did not show significant variations of the CD3⁺ T-lymphocyte content (65–70%). From 57 animals 6 animals became diabetic around day 60. All diabetic animals showed less than 40% of CD3⁺ T-lymphocytes. Microsatellite analysis revealed an association between the phenomenon of variant CD3⁺ T-lymphocytes and the microsatellite marker *D1Rat295* on the telomeric region of chromosome 1 (RNO1) with a LOD score of 11. The T-cell markers *Cd5* and *Cd6* are located in this region 300 kb proximal from the marker *D1Rat295*. Interestingly the microsatellite marker for the variant CD3⁺ T-lymphocytes mapped to the *Iddm8* locus (LOD score 4.1) at RNO1q51 and may therefore coincide with the development of T1DM in the LEW.1AR1-*iddm* rat.
**Conclusion:** The variant CD3⁺ T-lymphocyte content could be an additional indicator for the mutated gene locus which is responsible for the pathogenesis of diabetes in the LEW.1AR1-*iddm* rat. The genes *Cd5* and *Cd6* may play an important role for the selection or impaired elimination of autoaggressive cells in the LEW.1AR1-*iddm* rat. Perspectively the functional role of variant CD3⁺ T-lymphocytes may also help to understand mechanisms of autoimmunity in human T1DM.

# PS 11
# Candidate risk genes in Type 1 diabetes

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**Association of the vitamin D receptor (*VDR*) gene with Type 1 diabetes mellitus and celiac disease**

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**Background and aims:** The overlap between type 1 diabetes (T1DM) and celiac disease (*CD*) involves common genetic factors. Apart from the major locus in the HLA region on 6p21, there are a number of minor loci, some of which could be specific to one particular disease. One such candidate is the highly polymorphic vitamin D receptor (*VDR*) gene, also expressed in pancreatic β cells. Vitamin D has been shown to reduce lymphocyte activation, proliferation and cytokine production. Both diabetes and insulitis can be prevented in the NOD mouse by the administration of vitamin D or its analogues. Finally, epidemiological data suggest that vitamin D supplementation in childhood is associated with reduced T1DM incidence. Allelic variants of *VDR* have been studied in several diseases, including T1DM, with controversial results. The aim of this study was to determine whether *VDR* gene polymorphisms are associated with T1DM and CD and whether they share common susceptibility variants.
**Materials and methods:** A total of 64 families with T1DM and 37 CD families of Basque origin were genotyped for four *VDR* restriction-site polymorphisms (Fok I, Bsm I, Apa I and Taq I). Genomic DNA was extracted from peripheral blood, and PCR amplification, digestion with the appropriate restriction enzymes and agarose gel electrophoresis were performed as usual. The Affected Family Based Controls (AFBAC) approach was used to test for allele and haplotype associations. Additionally, 88 healthy controls were also analyzed and a case-control genotype analysis was performed.
**Results:**No single allele was associated with risk or protection from T1DM nor CD. AFBAC haplotype analyses showed a significantly higher frequency of haplotype *fBAt* in the T1DM group [$p_c$=0.02; OR=4.4 (1.5–15.3)]. Comparison of CD patients and healthy controls identified *ff* as a risk genotype [p=0.01; OR=3.45 (1.12–10.79)].
**Conclusion:** Polymorphisms within the vitamin D receptor gene are markers of susceptibility to autoimmune diseases. At least in Basque, association of *VDR* variants with T1DM and CD is heterogeneous, indicating that VDR polymorphisms are probably not the primary etiological genetic variants.

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**A novel polymorphism within the vitamin D receptor (VDR) is associated with Type 1 diabetes mellitus in Germans**

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**Background and aims:** The most active natural vitamin D metabolite 1,25 (OH)2D3, exerts its effects through binding to the vitamin D receptor (VDR). In vitro, 1,25 (OH)2D3 can modify antigen presentation and cytokine secretion and prevent the differentiation of T helper lymphocytes (Th) toward the Th1 subtypes, which are the main effector cells in type 1 diabetes mellitus. Additionally 1,25 (OH)2D3 can prevent the development of autoimmune diabetes in animal models.
**Materials and methods:** We analyzed the association of three novel polymorphisms A, B und C within exon 1 in the 5' UTR region of the VDR gene on chromosome 12 q12.
Families (n=171) comprising 513 individuals with one affected offspring with type1diabetes were genotyped for the three polymorphisms and transmission disequilibrium tests (TDT) were performed.
**Results:** We observed a positive association between one the polymorphism und type 1 diabetes mellitus. This polymorphism showed a significant transmission of the allele "b" (81 times transmitted vs. 48 times not transmitted) of heterozygous parents to affected offspring (PTDT= 0.0037), whereas the other two polymorphisms didn't demonstrate significant preferential transmission to affected offspring (PTDT= 0.2457 und 0.5764).
**Conclusion:** Our data show a significant association of the novel polymorphism B and type 1 diabetes mellitus in our population. The new polymorphism confirms our previous observations of an association of VDR with

type 1 diabetes mellitus. Further work is needed to confirm our findings and to difference the mechanisms by which variations in the VDR gene lead to variation in the cellular function.
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**The protein carboxyl methyltransferase gene and susceptibility for Type 1 diabetes**
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**Background and aims:** Isoaspartyl formation occurs spontaneously at susceptible sites in aging proteins and has been associated with autoimmunity. Protein Isoaspartyl *O*-methyltransferase (PIMT) is an enzyme that recognizes altered isoaspartyl residues in proteins converting them to normal aspartyl residues. Mice lacking PIMT have an autoimmune phenotype characterised by increased T-cell proliferation to mitogen and receptor-mediated stimulation, and wild-type mice to whom bone marrow from animals lacking PCMT is transferred, develop anti-nuclear auto-antibodies. The gene codifying PIMT; PCMT1 is located on the long arm of chromosome 6 (6q24-25), a region previously linked to the risk of type 1 diabetes (IDDM5).
**Material and methods:** A total of 26 affected members from an equivalent number of multiplex type 1 diabetes families demonstrating a linkage to markers on the long arm of chromosome 6 (IDDM5) were selected from the 147 Danish families included in the Scandinavian genome scan. Furthermore, 10 non-affected subjects were selected from 10 additional families, in which diabetes did not show linkage to this region. The 8 exons of the PCMT1 gene (1.6 Kb) were amplified (PCR) and sequenced (automated sequencing), as was the 800 bp 5' UTR region, presumed to have promoter activity.
**Results:** Variations in exon 5 (358G>A), in the 3' UTR in exon 8 (845A>G and 1072A>T) and in the 5'UTR region (-509C>G) were found in 24 subjects. These variations were in linkage disequilibrium with each other, giving rise to two haplotypes only. Previous studies have shown that the variation described in exon 5 leads to an aminoacid substitution (Val119/Ile199), and the presence of Ile in position 119 is associated with a higher activity of the PIMT enzyme to certain substrates. The variation described in the 5'UTR region leads to a change in a putative binding site (CAGC) for transcription factor Activator Protein 4, creating a new binding site (CAGG) for a different transcription factor: the Myoblast Determination Gene Product.
**Conclusions:** PCMT1, whose product is involved in protein repair, is a novel candidate gene for risk of type 1 diabetes. By sequencing 2.4 Kb of the PCMT1 gene, a functional variation has been detected, which will be explored for association with type 1 diabetes in larger family material.

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**Single nucleotide polymorphisms of the gene CYP24 are associated with Type 1 diabetes mellitus in Germans**
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**Background and aims:** CYP24 hydroxylase is a cytochrom P450 enzyme, which is encoded by a gene located on chromosome 20q13 and takes part in the vitamin D degradation cascade.
CYP24 gene itself was identified as a candidate oncogene in breast cancer. $1,25(OH)_2D_3$, the most active natural vitamin D metabolite effectively prevents the development of autoimmune diabetes mellitus and autoimmune thyroiditis in animal models. Given its immunological role we therefore investigated two novel singles nucleotides polymorphisms (SNPs) in the CYP24 hydroxylase gene for an association with type 1 diabetes mellitus in German families.
**Materials and methods:** One hundred five German families (315 subjects) with at least one affected offspring with type 1 diabetes mellitus were genotyped for the intronic polymorphism (T/C) and for the promoter polymorphism (C/G) within the gene of CYP24 hydroxylase and extended transmission disequilibrium tests (ETDT) were performed.
**Results:** A significant association was found between type 1 diabetes mellitus and allelic variation of the CYP24 polymorphisms. Our findings showed a higher transmission rate for the haplotype TG (50 times transmitted vs. 21 not transmitted, $P< 0.001$). On the other hand the haplotype CG was less often transmitted (23 times transmitted vs. 43 times not transmitted, $P < 0.02$). The ETDT haplotype-wise was $P = 0.0059$.
**Conclusion:** We found an association between type 1 diabetes mellitus und the CYP24 hydroxylase gene. This gene could be a new marker for this disease. Further genomic investigations in larger groups as well functional studies are underway to confirm our findings and to understand the role of the CYP24 hydroxylase with autoimmune type 1 diabetes mellitus.

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**Mutational screening of the human *CBLB* gene in individuals with several autoimmune diseases and evaluation of association to Type 1 diabetes**
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**Background and aim:** Recent studies of a spontaneous animal model of Type 1 Diabetes (T1D), the Komeda diabetes-prone (KDP) rat, have shown that the major part of the genetic disease susceptibility in the KDP rat is accounted for by the *MHC* region and a region containing the candidate gene, *Cblb* (Casitas-B-lineage lymphoma b). The KDP rat is characterized by autoimmune destruction of pancreatic beta cells, but in addition lymphocyte infiltration of other endocrine tissues is seen, indicating autoimmunity. A nonsense mutation in the *Cblb* gene in the KDP rat have been shown to be pathogenic. Cbl-b plays an important role in T-cell co-stimulation and is believed to be a negative regulator of autoimmunity. Dysregulation of Cbl-b may also contribute to autoimmune disease in man, and the aim of the present study was to screen the human *CBLB* gene for mutations of importance in autoimmune disease, and evaluate these by testing for association to T1D.
**Material and methods:** DNA from a material of 253 Danish T1D families (1097 individuals) was used initially. For replication another sample of 242 Danish T1D families (1027 individuals) were used. Mutational screening, by sequencing, was performed in a subset of 24 individuals with T1D and at least one other autoimmune disease, as well as 5 controls, without any autoimmune disease. Coding regions, exon-intron boundaries and 5´ and 3´UTR regions of the *CBLB* gene (Chr. 3q11-13.1) were screened. Verification of identified single nucleotide polymorphisms (SNPs) and estimation of minor allele frequencies were performed by genotyping a test-panel of 96 T1D individuals. Genotyping was performed by RFLP-PCR, mutagenically-seperated PCR or primer extension method. SNPs were tested for association to T1D by Sib-transmission-disequilibrium test. To evaluate the degree of linkage disequilibrium (LD) in the region, the pairwise LD between the SNPs in *CBLB* was evaluated. Parental haplotypes were estimated by maximum likelihood estimates in GeneHunter vs. 1.2, D´-values were calculated in HaploXT and results were visualized by GOLD software.
**Results:** Eight mutations were identified by sequencing, five existed in the NCBI SNP database (dbSNP), whereas three were novel. Two additionally coding SNPs in *CBLB* were identified from dbSNP. Two of these 10 SNPs had a minor allele frequency below 1% and were not tested further. Eigth remaining SNPs were genotyped in the material of 253 T1D families and tested for association to T1D. One SNP in Exon 12 was associated to T1D ($P = 0.0296$). We attempted to replicate this observation in another Danish T1D family material, consisting of 242 families, but were not able to confirm T1D association of the SNP. If pooled, though, 471 families were evaluated and association to T1D still observed ($P=0.0459$).
**Conclusion:** The *CBLB* gene is, based on animal models and its role in T-cell signaling and autoimmunity, an obvious functional candidate gene for autoimmunity in man. We demonstrated T1D association of a rare polymorphism in exon 12 of the human *CBLB* gene, and observed a high degree of LD in the region. Evaluation of the *CBLB* SNPs in samples of individuals suffering from several autoimmune diseases would be very interesting, as would studies of the eventual functional implications of the exon 12 SNP.

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**Convincing statistical support for association of the Arg620Trp polymorphism in *PTPN22* locus with Type 1 diabetes**

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**Background and aims:** Type 1 diabetes (T1D), a T-lymphocyte mediated autoimmune disease arising out of a complex interaction between genes and the environment. To date variation in the HLA, insulin and CTLA-4 genes have been associated with genetic predisposition to T1D. However, so far, linkage and association results in genetic analyses of common diseases indicates that large sample sizes are required to reliably detect loci with expected effects on disease predisposition, probably less than an odds ratios of 2.
**Materials and methods:** We used a large T1D family collection ($n$ = 2,434 families) and a new population-based case-control collection ($n$ = 4,000) to test the association of a recently described association of the lymphoid tyrosine phosphatase (PTPN22/LYP) gene in T1D.
**Results:** We were able to confirm the association of the Arg620Trp variant in this gene ($P = 1.4 \times 10^{-27}$) with T1D and observed this effect in several European populations.
**Conclusion:** However, this result does not imply that this single nucleotide polymorphism (SNP) is the causal variant and fine mapping of the region will be required to ensure that the Arg620Trp SNP is indeed the most associated variant in the region and that other potential functional disease-associated variants in the vicinity do not exist.

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**Intercellular adhesion molecule-1 gene polymorphisms in Type 1 diabetes**

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**Background and aims:** Nejentsev and colleagues (Lancet 2003, Nov 22, p 1857) in excellent study have recently shown in two large independent datasets that polymorphism G · A in exon 4 of ICAM-1 gene encoding G241R aminoacid substitutions in ICAM-1 molecule is associated with lower risk of type 1 diabetes. The aim of our study was to test the association of ICAM-1 gene polymorphism with type 1 diabetes in Polish population.
**Materials and methods:** Using PCR sequence specific primers method and direct DNA sequencing (ABI Prism 310) we have genotype polymorphisms in exon 4 (c.721G>A) and exon 6 (c1405A>G) of ICAM-1 gene in 211 Polish families affected by type 1 diabetes and 210 healthy controls from the same region of Poland.
**Results:** Similarly to Nejentsev et al. study we have observed less frequent transmission of 241R allele to affected offsprings from heterozygous parents (37.5%, 21 transmissions of 56, p=0,043) but also lower frequency of allele R in subjects with type 1 diabetes in comparison to healthy age matched controls (13.5% vs. 20.4%, p=0.008). We have also found the higher frequency of 469E allele in patients with type 1 diabetes in comparison to healthy controls (46.9% vs. 38.2%, p=0.01). Moreover we have analyzed the frequencies of alleles and genotypes in relation to the age-of diagnosis and we have observed that the frequency of 241R allele is a significantly lower in subjects with type 1 diabetes diagnosed before 25 yrs. of age in comparison to adult onset type 1 diabetes (11.8% vs. 21.6%, p=0.025).
**Conclusions:** We believe that our observations concerning the associations of ICAM-1 gene polymorphisms with the age of onset of autoimmune diseases give an additional support to the Nejentsev et al. hypothesis that potential ICAM-1 pathway-targeted treatment may delay the onset of type 1 diabetes mellitus.

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**Analysis of N-acetyl transferase 2 gene variants in Type 1 diabetes**

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**Background and aims:** Most cases of type 1 diabetes (T1D) are due to an immune-mediated destruction of the insulin-producing pancreatic beta cells, a process that is conditioned by multiple genes and environmental factors. N Acetyl Transferase (NAT) genes (chromosome 8p21.3-p23.1) are functional candidates for T1D, being involved in the conversion of dietary nitrates (which have been associated with risk of the disease) into diabetogenic N-nitroso compounds. We previously reported evidence for association of 590 G and 803 G alleles of NAT2 gene with T1D in 204 Romanian families ($p_{TDT}$ = 0.0075 for 590 G allele). The tested 590 G/A (rs1799930) and 803 A/G (rs1208) Single Nucleotide Polymorphisms (SNPs) of NAT2 gene are both coding, non-synonymous and influence the level of enzymatic activity. We tried to reconfirm these results by expanding the analysis to a larger cohort of European Caucasian families.
**Materials and methods:** The 590 G/A and 803 A/G polymorphisms were typed in 423 Romanian (including the original 204 and 219 new families), 457 UK, 231 USA, 250 Northern Ireland and 839 Finnish families. Genotyping was carried out using the Taqman® 5' nuclease assay and data were analysed by the Transmission Disequilibrium Test (TDT) using Stata® 8.1 (http://www.stata.com).
**Results:** We did not replicate our previous result on the 219 new Romanian families (54.31% transmission of 590 G allele to diabetics, $p_{TDT}$ = 0.35) even if the combined analysis of all 423 Romanian families showed a marginal effect of this allele (56.93% transmission, $p_{TDT}$ = 0.023). There was no evidence of allelic association of either SNP in any of the other populations and in the global analysis of all 2,280 families (50.55% transmission, $p_{TDT}$ = 0.59, respectively 49.25% transmission, $p_{TDT}$ =0.69).
**Conclusion:** Our results do not support an association of the tested SNPs with T1D, the weak association in the original Romanian population probably being a false positive. However, we cannot rule out an influence of NAT2 gene and more extensive analyses of additional polymorphisms in and near the gene will have to be carried out in the future. Before a final conclusion could be reached, it is also useful to analyse the NAT2 effect on T1D risk stratified according to the level of nitrate intake of diabetic patients in the years before the disease onset.

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**The GLU23Lys variant of the ATP sensitive K+ (K ATP) channel subunit is related to glycaemic control during the remission phase of young people with newly diagnosed Type 1 diabetes**

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**Background and aims:** Together with SUR1 the Kir6.2 constitute an ATP-sensitive potassium channel that is regulating glucose-dependent (via ATP) cellular activities ("glucose sensing") in pancreatic beta cells, enteroendocrine L cells in the distal gut and in neurones of appetite-regulating centers of the brain. The common Glu23Lys variant of Kir6.2 is less sensitive to ATP and is associated with type 2 diabetes and reduced serum insulin release during an oral glucose tolerance test in healthy subjects. The aim of the present study was to investigate whether the Glu23Lys variant also has influence on residual b-cell function, meal stimulated GLP-1 release and the overall glycaemic regulation during remission of 275 children and adolescents less than 16 years with newly diagnosed type I diabetes.
**Materials and methods:** A stimulated C-peptide and GLP-1 "Boost"-test (mixed meal) was carried out in each subject at 1, 6 and 12 months after diagnosis. Results: Genotyping for the Glu23Lys variant of the Kir6.2 and subsequent analyses by means of repeated measurement models showed that carriers of the variant had a tendency towards a reduced stimulated serum GLP-1 level (coefficient 0.11, p=0.087) while the stimulated serum C-peptide level was unchanged (p=0.85) compared to non carriers. Interestingly, HbA1 c (adjusted for insulin dosage), by repeated measurement

during 12 months period, was significantly (p=0.03) increased for those carrying the variant (coefficient 0.42).
**Conclusion:** The study indicate that the Kir6.2 variant might play a central role for glycaemic control of type 1 diabetes in remission by a combination of reduced ATP sensitivity in appetite regulating neurones of the brain (increased appetite), a faster intestinal absorption of nutrients and an increased postprandial glucagon level secondary to reduced GLP-1 release.

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**Strong association of the insulin gene locus (*IDDM2*) with Type 1 diabetes in the Romanian population**

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**Background and aims:** Type 1 diabetes mellitus (T1DM) is a common, chronic autoimmune disease that arises from the specific destruction of insulin secreting pancreatic β cells by autoreactive T lymphocytes. The pathogeny of T1DM is complex, involving the interaction of genetic and environmental factors. The insulin gene *INS*-VNTR region on chromosome 11p15 is one of the main susceptibility loci. We previously reported a very strong effect of Class I *INS*-VNTR alleles on T1DM risk for a sample of 204 Romanian families. Our aim was to reconfirm this association on a much larger cohort of Romanian T1DM families.
**Materials and methods:** The -23*Hph*I A/T SNP of the insulin gene (an accurate marker of the *INS*-VNTR alleles) was genotyped in 423 Romanian T1DM families (including the original 204 and 219 new families). The study group comprised 1515 individuals with 430 T1DM probands (206 M/224 F) and 1085 unaffected parents and siblings. Genotyping was done using the Taqman® 5' nuclease assay and data were analysed by the Transmission Disequilibrium Test (TDT) using Stata® 8.1 (http://www.stata.com).
**Results:** For the second Romanian dataset of 219 families, we found a significantly increased transmission of -23HphI A allele to diabetics (78.31% transmission, $p_{TDT}$ = 2.4 e-07) which confirms our previous findings. Combined with the results from the first 204 families, the transmission of -23HphI A allele to diabetics is almost 80% (79.78%, $p_{TDT}$ = 2.8 e-15). These data indicate almost the same level of predisposition as for the most diabetogenic HLA's in the Romanians (DQB1*0302 and DQB1*02).
**Conclusion:** We found an exceptionally strong association of the diabetogenic *INS*-VNTR alleles with T1DM in Romania. The association is stronger than that reported in other Caucasian populations and, at least on this sample of the Romanian population, comparable with that of the most diabetogenic HLA's.

# PS 12
# Candidate risk genes in Type 2 diabetes

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**Haplotype within the promoter of the APM1 gene linked to the progression toward Type 2 diabetes through a pre-existing hypoadiponectinaemia, in subjects from diabetes pedigrees**

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**Background and aims:** Adiponectin, encoded by the *APM1* gene has been shown to modulate insulin sensitivity and glucose homeostasis. Plasma adiponectin levels are decreased in type 2 diabetes and obesity. Recent investigations have led to the elucidation that genetic variation within the *APM1* gene is associated with decreased adiponectin hormone levels. The aim of this investigation was to analyse genetic variation within *APM1* in relation to adiponectinaemia and the progression toward type 2 diabetes.
**Materials and methods:** 550 probands with a familial history of type 2 diabetes were included in this investigation. All probands underwent a 75g oral glucose tolerance test following an overnight fasting period, with measurements of plasma glucose, insulin, C-peptide, proinsulin and free fatty acids determined at fasting and at 30, 60, 90 and 120 minutes proceeding glucose challenge. Utilising the ADA/WHO criteria, probands were assigned into groups of normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and type 2 diabetes (T2DM). 3 common SNPs in the *APM1* gene, namely +45T>G in exon 2, and 2 promoter variants SNPs -11391G>A and -11377C>G were genotyped via real time polymerase chain reaction and fluorescence resonance energy transfer (FRET) technology.
**Results:** The -11391 G and -11377 G were associated with lower adiponectin levels (p=0.0001 and p=0.02, respectively) but no association was detected with the +45TG (p=0.6). Analysis after three years produced similar results. A strong association between the evolution of the disease and adiponectin levels was determined (p<0.0001). Carriers of the wild / " high level haplotype " W/H haplotype combination at the end of the 3 year follow up had a twofold decreased diabetes risk, OR=0.42, [95%CI 0.17-.94] p=0.04. The area under the curve for free fatty acids was significantly lower for the individuals bearing this haplotype combination than compared to the remaining subjects at first visit (p=0.04) and after three years (p=0.019).
**Conclusion:** The investigation provides evidence that genetic variation in the adiponectin (*APM1*) gene promoter region is associated with increased diabetes risk in a German Caucasian population and replicates previously reported associations. The investigation provides additional evidence that evolution of the disease is correlated with pre-existing adiponectin levels at inclusion, rather than with the variation of adiponectin levels during the evolution of the disease. The risk of T2D is decreased for individuals harbouring the "high adiponectin level" genetic configuration at the *APM1* promoter. The same haplotype is associated with lower FFA levels both at inclusion and after the 3 year interim period. From the current investigation, it was determined that there is a correlation between genetic variants at the APM1 promoter, adiponectin levels, insulin sensitivity as assessed by FFA levels, as well as the evolution of the diabetic status.
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**A novel polymorphism in the adiponectin receptor gene, AdipoR1, is strongly associated with Type 2 diabetes**

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**Background and aims:** The adipose-specific protein, adiponectin, appears to have a major role in the pathogenesis of insulin resistance and type 2 diabetes. Two adiponectin receptors, AdipoR1 and AdipoR2, bind globular and full-length adiponectin and activate the signaling molecules PPAR-alpha, AMPK and p38MAPK to potentiate the effects of adiponectin.

Human AdipoR1 is located on chromosome 1p36.13-q41 and AdipoR2 is located on chromosome 12p13.31. This study aimed to determine whether single nucleotide polymorphisms (SNPs) and other polymorphisms exist within the exon sequences of adiponectin receptor genes.
**Materials and methods:** The 13 exons within the adiponectin receptor genes, AdipoR1 and AdipoR2, were amplified by PCR and sequenced using ABI protocols on an ABI1300 DNA sequencer in 20 individuals. Two novel polymorphisms have been identified in the promoter region of AdipoR1 (-106A>G and -02T>G). Sequencing revealed that the polymorphisms were in 100% linkage disequilibrium. In preliminary work the frequency of these polymorphisms was determined in young healthy control subjects of normal body mass (n=87, 28 males) and in individuals newly-presenting with type 2 diabetes (n= 49, 25 males, mean age 56.0 ± 1.4 y, mean BMI 31.0 ± 0.9 kg/m$^2$, mean HbA1 c 5.7 ± 0.1%).
**Results:** The -106G polymorphism was observed in 72% of control individuals (30% of control individuals were homozygous) and 88% of type 2 diabetes cases (46% were homozygous). The -106G allele was significantly associated with type 2 diabetes (OR=2.73, 95% CI: 1.03–7.25, p=0.038). Computer predictions indicate that the G allele contains multiple overlapping transcription factor sites, which are not present in the A allele, suggesting that the polymorphism is associated with altered expression of the receptor.
**Conclusion:** This newly-identified polymorphism in the promoter region of the AdipoR1 gene is likely to result in functional changes that may predispose individuals to obesity and type 2 diabetes.

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**Adiponectin-genotype affects obesity-related diabetes risk and circulating adiponectin levels: Results of the two independent cohorts EPIC-Potsdam and MesyBepo**

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**Background and aims:** About 70% of obese individuals do never experience type 2 diabetes in their lifetime, although obesity is a major risk factor for development of type 2 diabetes. Adiponectin is an insulin-sensitizing and putative anti-inflammatory cytokine, which is reduced in obesity. Interestingly, linkage studies revealed a susceptibility locus for type 2 diabetes on chromosome 3q27, where the adiponectin-gene is localized. Results of recent association studies were partially controversial with respect to investigated polymorphisms, although some studies were suggestive that the common adiponectin promoter polymorphism -11377 G/C might be associated with T2DM.
**Materials and methods:** We thus aimed to investigate the interplay between this potentially relevant genetic polymorphism and environmental factors with respect to adiponectin plasma levels and diabetes risk in a 2.3-year prospective, nested case-control study (n=564) based on the EPIC-Potsdam cohort with more than 27,000 individuals. We additionally analyzed a second independent cross-sectional cohort of healthy individuals from the region of Berlin (n=245) with respect to diabetes-associated quantitative traits and adiponectin plasma levels.
**Results:** We demonstrate in the prospective EPIC-Potsdam cohort a significant interaction between the common adiponectin -11377 G/C SNP and BMI with respect to diabetes risk. Additionally the -11377 G/C SNP affects circulating adiponectin levels in this cohort. This association between the -11377 genotype and adiponectin levels was confirmed in a second independent cohort. Additionally a significant effect of the -11377 G/C SNP on basal insulin levels was found in this second cohort even after adjustment for age, sex and total body fat.
**Conclusion:** Our results support the assumption that adiponectin is involved in the development of type 2 diabetes. In more general terms, we claim that integration of environmental data should be mandatory in genetic analyses of complex diseases.

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**The polymorphisms in the promoter region of fatty acid binding protein 2 are associated with Type 2 diabetes**

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**Background and aims:** Intestinal fatty acid binding protein (FABP2) is an cytosolic protein in small intestine epithelial cells. FABP2 has a high affinity for long-chain fatty acids and participates in absorption of dietary fatty acids. Recently, insertion-deletion polymorphisms in the promoter of *FABP2* was discribed in two studies. As found by reporter assays *in-vitro*, these polymorphisms affected promoter activity. The more active promoter variant B was associated with a delayed postprandial increase of triglycerides and lower body mass index in non-diabetic subjects. Because these parameters contribute to type 2 diabetes, we performed an association study for testing whether the promoter polymorphisms were associated with the risk of type 2 diabetes.
**Materials and methods:** Subjects were taken from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study. 192 incident cases of type 2 diabetes were identified and medically proven by confirmation of the primary care physician. Cases were matched with 2 control subjects each by age and sex (n=384). The region 5' of the initiation codon of the *FABP2* gene contains three insertion/deletion polymorphisms: a T insertion at -80bp, an AGTAG deletion at -136bp and an AAG>T deletion at -166bp. The polymorphisms above were in a complete linkage disequilibrium. Thus, two haplotypes existed for the three polymorphisms named allele A and B. We genotyped two of the polymorphisms: -166 AAG>T und -80 T deletion in the subjects. Genotyping was performed by using the pyrosequencing method.
**Results:** The allele frequencies in the whole study population were 57.91% for the allele A and 42.09% for the allele B. The genotype distribution was in compliance with the Hardy-Weinberg equilibrium (p=0.510). Although the allele B was less frequent in cases (0.395) than in controls (0.434) as well as the B/B genotype (cases: 0.136; controls: 0.188), the differences were not statistically significant. After adjusting for BMI by conditional logistic regression, *FABP2* B/B genotype was associated with significantly decreased risk of type 2 diabetes compared to A/A genotype. The relative risk was 0.455, 95% CI=0.238–0.870, p=0.017.
**Conclusion:** Our result suggests that the allele B in the *FABP2* promoter is associated with a decreased risk of type 2 diabetes.
*Supported by: BMBF*

## 359

**The Leu72Met polymorphism of the ghrelin gene predicts the conversion from impaired glucose tolerance to Type 2 diabetes**

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**Background and aims:** Ghrelin is a gut-brain regulatory peptide stimulating appetite and controlling energy balance. In previous studies the Leu72Met polymorphism of the ghrelin gene has been shown to be associated with obesity in adults and children as well as with decreased insulin secretion in obese children. We investigated possible associations of the Leu72Met polymorphism with the incidence of type 2 diabetes in subjects with impaired glucose tolerance (IGT) in the Finnish Diabetes Prevention Study.
**Materials and methods:** The Finnish Diabetes Prevention Study is a longitudinal study carried out in five participating centres in Finland. Altogether, 522 subjects with IGT were randomised into either an intervention or control group. DNA was available from 507 subjects (mean BMI 31.2 ± 4.5 kg · m$^{-2}$, age 55.3 ± 7.1 years). The Leu72Met polymorphism was screened by RFLP-method.
**Results:** Genotype frequencies in the entire study population were 76.9% for Leu72Leu, 19.7% for Leu72Met and 2.7% for Met72Met. There were no differences in baseline characteristics among the genotypes. The presence of the 72Met allele was associated with the 3-year incidence of type 2 diabetes in all subjects (p = 0.040, OR = 1.78, 95% CI 1.03–3.08), and in the intervention group separately (p = 0.015, OR = 3.18, 95% CI 1.26–8.04), but not in the control group (p = 0.509, OR = 1.27, 95% CI 0.63–2.56). Adjustment for baseline fasting blood glucose level, baseline weight and weight change did not change the results.

**Conclusions:** The Leu72Met polymorphism of the ghrelin gene may be a genetic risk factor for the conversion from IGT to type 2 diabetes.
*Supported by: Academy of Finland*

## 360

**Interactions of the variants of β3-(Trp64Arg) adrenergic-receptor and uncoupling protein-2 (Ala55Val) genes on obesity and Type 2 diabetes in Chinese**

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**Background and aims:** Modest associations between obesity and diabetes and the $\beta_3$-AR gene and the UCP2 gene have been reported. We aimed to investigate the synergic effects of $\beta_3$-AR gene Trp64Arg variation and UCP2 gene Ala55Val variation on obesity and type 2 diabetes in Chinese Han population.
**Materials and methods:** A total of 469 Chinese subjects included 119 obese (BMI 27.9 ± 2.98 kg/m$^2$), 173 type 2 diabetics (BMI 21.9 ± 2.0 kg/m$^2$) and 177 control subjects (BMI 21.9 ± 1.9 kg/m$^2$) were genotyped for the Trp64Arg variant in the $\beta_3$-AR and for the Ala55Val variant in the UCP2 genes using PCR-RFLP. The effects of each gene variant singly and jointly were estimated as fixed effects using the measured genotype approach framework. Statistics significance of frequency differences was evaluated by chi-squared test analysis and the non-parametric test was employed to compare among the three groups, and the synergic effects of the both gene variations were analyzed.
**Results:** (1) The frequency of homozygote of $\beta_3$-AR gene Trp64Arg variation in diabetics was higher than that in control subjects (OR=9.63, *P*=0.01). The Trp64Arg variation carriers had higher fasting and 2-hour post-prandial glucose levels than non-carriers in control subjects. (2) The frequency of homozygote of UCP2 gene Ala55Val variation in diabetes and obesity were higher than the control subjects (OR=4.62, *P*=0.001;OR=3.71, *P*=0.001). The Ala55Val variation carriers in control subjects had higher BMI. (3) The frequency of variants in diabetics and obese subjects with only UCP2 gene or $\beta_3$-AR gene mutation was the same as in control subjects (*P*>0.05). In case of concomitant UCP2 gene and $\beta_3$-AR gene mutations, the frequency was higher in diabetics and obese patients than in control subjects (OR=3.69, *P*=0.002; OR=2.57, *P*=0.009). (4) The Val/Val+ Trp/Arg carriers had the greatest risk to develop diabetes (OR=10.43, *P*=0.000) and obesity (OR=8.58, *P*=0.002). The Ala/Val + Trp/Arg was relation with diabetes (OR=2.55, *P*=0.000).
**Conclusion:** The homozygote of $\beta_3$-AR gene Trp64Arg mutation was related with diabetes. The homozygote of UCP2 gene Ala55Val mutation increases the risk of diabetes and obesity. The Val/Val + Trp/Arg carriers had the greatest risk to develop diabetes and obesity. The present study demonstrates that the progression of two micro-genes had the effect of promoting the occurring of disease.

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**Association between obese Type 2 diabetes and the melanocortin 2 receptor gene (*MC2R*) on chromosome 18p11**

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**Background and aims:** Linkage between obese type 2 diabetes (OBT2D) and chromosome 18p11 has been reported in Finnish, Dutch and Icelandic genome-wide-scans, and gender specific differences have been suggested. Our earlier screening of positional candidate genes on 18p11 identified a polymorphism (SNP2, T/C) in the *MC2R* promoter, which was associated with OBT2D in the linked families. First aim of the present study was to test whether association between OBT2D and SNP2 can be replicated in another case-control population from Finland and to investigate whether the association is gender specific. Second, we studied phenotypic differences between genotype-discordant sibling pairs originating from families with T2D and obesity
**Materials and methods:** SNP2 was genotyped in 201 T2D patients (m/f 84/117, age 61 ± 12 y, BMI 29.8 ± 4.6 kg/m$^2$) and 118 control individuals (m/f 58/60, age 60 ± 9 y, BMI 27.2 ± 4.1 kg/m$^2$) and in 534 siblings from 88 families with at least 2 siblings with T2D and BMI>30 kg/m$^2$. Of the 201 T2D patients, 89 had BMI>30 (m/f 36/53, age 59 ± 11 y, BMI 33.8 ± 3.3 kg/m$^2$) and 93 controls had BMI<30 (m/f 48/45, age 60 ± 9 y, BMI 25.7 ± 2.6 kg/m$^2$). Phenotypic differences (age, BMI, waist circumference, waist-to-hip ratio, fasting plasma glucose, fasting plasma insulin, HDL-cholesterol and triglycerides) between the genotype-discordant sibling pairs were analyzed by permutation test (100 000 permutations) and differences in allele- and genotype frequencies were tested by chi-square analysis.
**Results:** Allele- and genotype frequencies did not significantly differ between T2D patients and control individuals when the data were not stratified for BMI or gender (p=0.14 and p=0.11, respectively). Among females, the common TT genotype was associated with T2D (91.0 vs. 78.3%, p=0.033), particularly after BMI stratification (95.9 vs. 75.6%, p=0.0043). Among males, no significant differences in allele- or genotype frequencies could be detected between the groups. Totally 79 genotype-discordant sibling pairs were identified including 87 unique individuals from 19 families containing 32 T2D pairs, 16 female- and 17 male pairs. Among female pairs individuals carrying the TT genotype had higher BMI (32.8 ± 5.4 vs. 29.4 ± 4.7 kg/m$^2$, p=0.027) and higher waist circumference (103 ± 12 vs. 94 ± 11 cm, p=0.026) compared to their sisters with the other genotypes. While BMI did not significantly differ between male sibling pairs, males with the TT genotype had elevated triglyceride levels (2.9 ± 2.5 vs. 1.3 ± 0.4 mmol/l, p=0.011) compared to their brothers with the other genotypes.
**Conclusion:** We could replicate the association between the *MC2R* TT genotype and OBT2D in females and found that in families with OBT2D, females with the TT genotype had higher BMI and waist circumference compared to their siblings without this genotype. Our results further support a role for *MC2R* in OBT2D and suggest gender specific differences in OBT2D susceptibility by this locus. Functional studies of the potential consequences of *MC2R* SNP2 in adrenocortical and adipose tissue are undertaken and will be discussed.

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**Putative association between variants in the *GPR40* gene and Type 2 diabetes**

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**Background and aims:** The G protein-coupled receptor GPR40/ $FFA_1R$ is the first gene product identified to act as an extracellular membrane receptor for free fatty acids (FFAs) in pancreatic islets. It was recently shown to be activated in beta cells *in vitro* by medium and long chain free fatty acids (FFAs) as well as by the antidiabetic thiazolidinedione drugs, causing elevated $Ca^{2+}$ concentrations and subsequent promotion of insulin secretion. The *GPR40* ORF encompasses 903 bp encoded from a single exon and is expressed mainly in pancreatic beta cells but also in brain in humans. These properties suggest that GPR40 could be a mediator of lipotoxicity, rendering it as a suceptibility gene for human type 2 diabetes (T2D).
We studied whether variation in the *GPR40* locus is associated with type 2 diabetes by re-sequencing the *GPR40* gene and genotyping identified SNPs in patients with type 2 diabetes and control subjects from the Botnia study in Finland.
**Materials and methods:** In the first part, a 2379 bp long DNA sequence including the 903 bp of the coding region in the GPR40 gene was sequenced in 48 T2D (31 male, 17 females) and 48 healthy glucose-tolerant control subjects (20 males, 28 females).
We identified 13 SNPs, and two of them at positions 598 and 597 bp upstream the transcription initiation site, were chosen for genotyping using the SNaPshot assay in a larger scale – 499 T2D patients (243 males, 256 females, age = 66 years, BMI = 28.4 ± 4.6 kg/m$^2$, age at onset = 57 years) and 259 control subjects (126 males, 133 females, age = 54 years, BMI = 25.4 ± 3.6 kg/m$^2$). Genotype frequencies were compared by chi-square test whereas differences in insulin secretion (incremental insulin and insulinogenic index) between different genotype carriers – using one-way ANOVA test.
**Results:** 13 SNPs were identified by sequencing, two of them were synonymous SNPs within the coding region. Four of them represented dbSNPs in the NCBI database (www.ncbi.nlm.nih.gov). Neither of two SNPs showed statistically significant association with T2D-genotype frequencies at SNP position 598 were as follows: T/T=33.33%, T/C=50.14%, C/C=16.53% in T2D patients and T/T=39.68%, T/C=45.75%, C/C=14.57% in control subjects (p=0.27), whereas at SNP position 597: A/A=29.97%, A/G=51.82%, G/G=18.21% in T2D patients and A/A=30.24%, A/G=44.35%, G/G=25.40% in control subjects (p=0.072).
However, the insulinogenic index was lower in carriers of the T allele assuming a dominant model (T/T+T/C, 25.34 ± 40.95 versus C/C, 45.19 ± 36.04; p=0.011) at SNP position 598 and for G allele (G/G+A/G, 22.24 ± 28.38 versus A/A, 48.78 ± 40.43; p<0.0001) at SNP position 597 in control subjects.
**Conclusions:** The results suggest the SNPs in the *GPR40* gene might influence insulin secretion.

# PS 13

# Functional genetics in Type 2 diabetes

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**Alterated response to hyperglycemia-derived oxidative stress in diabetic patients with eNOS Glut298Asp polymorphism**

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Nitric oxide is produced by nitric oxide synthase enzymes (NOS), endothelial NOS (eNOS), neuronal (nNOS), and inducible NOS. eNOS, constitutively expressed enzyme, produces low levels of NO, necessary for the integrity and correct function of endothelium. In diabetic patients hyperglycemia-dependent superoxide overproduction quench nitric oxide, this process leads to formation of peroxinitrite, a strong oxidant product which in turn is responsible of endothelial dysfunction and initiates protein nitration. The production of peroxynitrite can be indirectly inferred by the presence of nitrotyrosine. Functional polymorphisms in the gene that encodes eNOS have the potential to affect disease development. The last known polymorphism, lies within the open reading frame, located at exon 7, Glu298Asp leads to primary structure alteration of the protein. This polymorphism of eNOS has been recently associated with abnormal vasorectivity, ischemic heart disease and myocardial infarction.
**Aim of the study:** We evaluated if diabetic patients with type 2 diabetes, omozygotic for Glu298Asp polymorphism of eNOS, shown alterated response to oxidative stress generation after assumption of standard meal.
**Materials and methods:** 40 patients, with type 2 diabetes, assumed a standard meal. At time 0, 60', 120' e 180' were evaluated glycemia and oxidative stress levels, with determination of nitrotyrosine (NT). Moreover we sottoposed all the patients to genetic screening for the research of Glu298Asp polymorphism of eNOS, this leads to subdivided patients in three groups: omozygotic for Glu298Asp mutation (6 patients), normal omozygotic (14 patients) and eterozygotic (20 patients). We evaluated variation of glycemia and nitrotyrosine after meal in the three groups.
**Results.** Increased glycemia values during meal, calculated in percent of variation respect to basal values, weren't significantly different in the three groups. Increased nitrotyrosine values, calculated in percent of variation respect to basal values, were significantly different ($p<0,05$) in omozygotic Glu298Asp group respect to normal omozygotic group at all time (T60': 42,1%± 17,7 vs 23,1%± 16,4; T120': 41,1%± 20,4 vs 22,6%± 14,7; T180': 36,8%± 18,3 vs 15,7% ± 10,0)(variation percent±DS). Moreover it was significantly different ($p<0,05$) between omozygotic Glu298Asp and eterozygotic at 60' (42,1%± 17,7 vs 22,8%± 16,5).
**Conclusions:** This study have shown that diabetic patients, omozygotic for polymorphism Glu298Asp of eNOS, present alterated response to hyperglycemia-derived oxidative stress.

## 364

**Quantitative trait loci near the insulin-degrading enzyme gene (*IDE*) contribute to variation in plasma insulin levels**

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**Background and aims:** Insulin degrading enzyme plays a principal role in the proteolysis of several peptides in addition to insulin and is encoded by *IDE*, which resides in a region of chromosome 10q linked to type 2 diabetes mellitus (T2DM). Two recent studies presented genetic association data on *IDE* and T2DM (one positive and the other negative). The present study was designed to explore the fundamental question of whether polymorphism in *IDE* has a measurable influence upon insulin levels in human populations.
**Materials and methods:** Fourteen single nucleotide polymorphisms (SNPs) from a linkage disequilibrium (LD) block encompassing *IDE* have been genotyped in a sample of 321 impaired glucose tolerance (IGT) and 403 non-diabetic control subjects in Swedish Caucasians by using dynamic allele specific hybridization (DASH).
**Results:** Analyses based upon haplotypic genotypes (diplotypes), constructed with SNPs that differentiate common extant haplotypes extending across *IDE*, provided compelling evidence of association with fasting insulin levels ($P=0.0009$), 2h insulin levels ($P=0.0027$), HOMA-IR ($P=0.0001$), and BMI ($P=0.0067$), with effects exclusively evident in men. The strongest evidence for an effect of a single marker was obtained for rs2251101 (located near the 3'-UTR of *IDE*) upon 2h insulin levels ($P=0.000023$).
**Conclusion:** Polymorphisms in *IDE* contribute to a large proportion of variance in plasma insulin levels and correlated traits. However, questions of gender specificity and allelic heterogeneity will need to be taken into consideration as the molecular basis of the observed phenotypic effects unfolds.

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**Fatty Acid Binding Protein 2 promoter polymorphism is associated with insulin sensitivity after a mixed meal in the Metabolic Intervention Cohort Kiel**

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**Background and aims:** The fatty Acid Binding Protein (FABP2) participates in the absorption of dietary fats. FABP2 KO mice have higher triglyceride levels and hyperinsulinemia. The single nucleotide polymorphism (SNP) in exon2 of the FABP2 gene results in an amino acid switch (Ala-Thr) and is associated with increased insulin levels. Recently we have detected SNPs in the promoter region resulting in two alleles: A and B. Allel B was associated with increased activity and was negative associated with type 2 diabetes. The aim was to investigate the association between SNP and postprandial metabolism and phenomenologic parameters in the Metabolic Intervention Cohort Kiel.
**Materials and methods:** 750 men, 45 to 65 years old, without a diagnosis of diabetes were recruited for a standard glucose tolerance test and the postprandial assessment of metabolic parameters after a standardized mixed fat meal (51.6 kcal% fat, 29.6 kcal% carbohydrates, 11.9 kcal% protein). The promoter region of the FABP2 gene contains three insertion/deletion polymorphism, wich are in linkage disquilibrium resulting in two haplotypes: T insertion at -80bp, an AGTAG deletion at -136bp, and an AAG>T deletion at -166bp. We genotyped two of these polymorphisms by using pyrosequencing method.
**Results:** The genotype frequency was for allele AA: 0.21, AB: 0.54 and BB: 0.24. No significant difference between groups were found in respect to BMI, WHR, waist circumference, fasting serum TG, postprandial TG, LDL and total cholesterol. When calculated in a dominant model (BB + AB) we found significant difference for fasting HDL (mean±S.E.M) (variant BB + AB: 54.1 ± 0.619 mg/dl variant AA:51.3 ± 1.14 mg/dl; $p<0.05$). After the mixed meal significant difference was found for postprandial insulin levels (AUC) (BB+AB:195.6 ± 5.99 µU/ml; AA:228,0 ± 17.2 µU/ml; $p<0.05$) as well as the postprandial product of glucose and insulin (AUC) (BB+AB: 1276 ± 48,9 µU/ml*mmol/l*h, AA: 1549 ± 162,2 µU/ml*mmol/l*h; $p<0.05$) whereas the difference after glucose ingestion was less pronounced. Further we have seen a significant difference between groups for weight increase within the last 20 years (AA: 16.6 ± 2.2 kg; AB: 14.9 ± 1.12 kg; BB: 10.48 ± 1.67 kg; $p<0.05$).
**Conclusion:** The promoter variant B of FABP2 was associated with fasting HDL-plasma levels and a higher postprandial insulin sensitivity. These findings support the assumption that this promoter variant may protect from type 2 diabetes.
*Supported by: Academy of Finland*

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**Mutations of transglutaminase 2 and early onset Type 2 diabetes**

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**Background and aims:** Transglutaminase 2 (TGase 2) is a $Ca^{2+}$-dependent enzyme catalyzing cross-linking reactions through transamidation of spe-

cific glutamine residues. TGase2 is the only member of TGase family to have ubiquitous expression, while other TGases have tissue specific expression. Furthermore, TGase 2 can bind and hydrolize GTP. In pancreatic β cells TGase 2 is involved in glucose-dependent insulin secretion. We have recently observed that TGase $2^{-/-}$ mice are phenotypically similar to "Maturity Onset Diabetes of the Young" (MODY) patients and through genetic screening of 23 Italian MODY patients we have identified a heterozygous missense mutation (N333S) in the catalytic site of the enzyme. These data supported the concept of Tgase2 as a potential candidate gene in MODY or Type 2 Diabetes.
**Materials and methods:** We have analysed the complete coding sequence of TGase 2 gene in 167 patients affected by MODY or early onset type 2 diabetes (age<40 years): 48 patients were collected in Italy, 87 in Denmark, 20 in UK, and 12 in Norway. The 12 exons of TGase 2 gene were amplified by PCR and analysed through "denaturing High Pressure Liquid Chromatography" (dHPLC). DNA of samples with anomalous chromatographic profile were automatically sequenced. Identified mutations were introduced by site-directed mutagenesis in the pSG5 plasmid containing the human TGase 2 cDNA and transamidating activity assessed through $H^3$-spermidine incorporation after transient transfection of COS7 cells.
**Results:** In one Danish patient we identified the heterozygous mutation T989 → G which determines the substitution of methionine in position 330 with arginine (M330R). Mutation M330R was not observed in 100 Danish control subjects. We have introduced N333S and M330R mutations in pSG5-TGase2 plasmid (pSG5-N333S e pSG5-M330R) and assessed TGase activity in COS7 transiently transfected with pSG5-WT, pSG5-N333S, pSG5-M330R and pSG5-SER (constitutively inactive) plasmids. TGase activity in COS7 cells transfected with pSG5-WT was 18 fold higher than in native COS7, while pSG5-SER had no effect on TGase activity. Cells expressing pSG5-N333S and pSG5-M330R plasmids showed a reduced TGase activity (59.4%± 2.7 and 50.1%± 4.8, respectively for pSG5-N333S and pSG5-M330R) compared with pSG5-WT transfected cells. Real Time PCR experiments showed that TGase 2 is the only enzyme of TGase family that is significantly expressed in human pancreatic islets. Preliminary data also indicate that a measurable transamidating activity is present in human pancreatic islets.
**Conclusion:** Our data suggest a role of N333S and M330R mutations in impairing insulin secretion in carriers and that TGase 2 might play a role as a modulator of β-cell function.

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**The interleukin-6 gene alleles -598G, -573C and -174G are associated with Type 2 diabetes and with modulations of systemic immune mediator concentrations**

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**Background and aims:** During the past two years, overwhelming evidence accumulated that a subclinical inflammation plays a crucial role in the pathogenesis of type 2 diabetes (T2D). Elevated blood concentrations of interleukin-6 (IL-6) have been demonstrated to be one of the most relevant immunological risk factors for T2D. Since there are currently no data available for the association of the single nucleotide polymorphisms (SNPs) A-598G, C-573G and C-174G in the IL-6 promoter with diabetes status, components of the metabolic syndrome and low-grade systemic inflammation, we analyzed the impact of the three SNPs on these parameters in the population-based KORA Survey 2000.
**Materials and methods:** The KORA (Cooperative Research in the Region of Augsburg) Survey 2000 studied a population-based sample of 4,261 subjects aged 25–74 years during the years 1999–2001. In the age range of 55–74 years 1,653 persons participated in a standardized interview followed by biochemical and clinical analyses. A total of 230 individuals with T2D and 235 individuals with impaired glucose tolerance (IGT) were available for analyses. 239 normoglycemic controls were randomly selected after matching for age and sex.
**Results:** Conditional logistic regression showed that the -573C allele was significantly associated with T2D (OR 2.03, $P = 0.030$). Heterozygous carriers of the C allele had higher levels of circulating IL-6. The -598G and -174G alleles were in strong linkage disequilibrium and also significantly associated with T2D (OR 1.49, $P = 0.010$ and OR 1.48, $P = 0.010$, respectively). The association was stronger in men and in leaner subjects (BMI lower than 28.7 kg/m$^2$). Circulating IL-6 levels were not affected, but significantly elevated levels of the chemokine CCL2/MCP-1 in carriers of the protective genotypes indicated an indirect effect of these SNPs.
**Conclusion:** Taken together, our findings indicate that different SNPs of the IL-6 gene influence diabetes risk by different mechanisms. The -598G/-174G alleles confer increased risk of T2D in men with lower BMI, probably by regulating MCP-1 levels. By contrast, the -573C allele might increase risk of T2D by affecting systemic IL-6 levels.
*The KORA Group consists of H.E. Wichmann (speaker), H. Löwel, C. Meisinger, T. Illig, R. Holle, J. John and their co-workers who are responsible for the design and conduct of the KORA studies.*

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**Impaired DNA repair and increased sensitivity to mutagens in Type 2 diabetes mellitus – a contribution to the association between diabetes and cancer?**

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**Background and aims:** DNA damage may be associated with type 2 diabetes mellitus (T2DM) and its complications mainly through the oxidative stress. Little is known about DNA repair disturbances potentially contributing to the overall extent of DNA damage in T2DM, which, in turn, may be linked with genomic instability resulting in cancer. To assess whether DNA repair may be perturbed in 2DM we determined: 1) the level of endogenous basal, oxidative and alkylative DNA damage 2) the sensitivity to DNA-damaging agents hydrogen peroxide and doxorubicin and the efficacy of removing of DNA damage induced by these agents in peripheral blood lymphocytes of T2DM patients and healthy individuals.
**Materials and methods:** The level of DNA damage and the kinetics of DNA repair was evaluated by the alkaline single cell gel electrophoresis (comet assay). Oxidative and alkylative DNA damages were assayed with the use of DNA repair enzymes endonuclease III (Endo III) and formamidopyrimidine-DNA glycosylase (Fpg), recognizing oxidized DNA bases and 3-methyladenine-DNA glycosylase II (AlkA) recognizing alkylated bases.
**Results:** The levels of basal endogenous and oxidative DNA damage in diabetes patients were higher than in control subjects ($p < 0.05$). There was not difference between the level of alkylative DNA in the patients and the controls. Diabetes patients displayed higher susceptibility to hydrogen peroxide and doxorubicin ($p < 0.05$) and decreased efficacy of repairing DNA damage induced by these agents than healthy controls.
**Conclusion:** Our results suggest that type 2 diabetes mellitus may be associated not only with the elevated level of oxidative DNA damage but also with the increased susceptibility to mutagens and the decreased efficacy of DNA repair. These features may contribute to the link between diabetes and cancer and metrics of DNA damage and repair, measured by the comet assay, may be considered as markers of risk of cancer in diabetes.
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**A single hyperglycemia QTL can cause diabetes in the presence of genetically induced extreme obesity**

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**Background and aims:** The common disease such as type 2 diabetes results from complex interplay among multiple genetic as well as environmental factors. Therefore, the genetic dissection is often hampered by rather weak effect from a single QTL or mutation. Consistently, our congenic rats carrying single diabetic QTL derived from the OLETF (Otsuka Long-Evans Tokushima Fatty) rat, age/obesity-dependent polygenic type2 diabetes model, show significantly high, but only mild hyperglycemia phenotype at 30 weeks of age. We introduced leptin receptor mutation into these congenic strains in order to encourage more manifest expression of diabetic QTLs.
**Materials and methods:** Male Zucker Fatty rat (SLC, Japan) was mated with F344 females to obtain F1 progeny, which was subsequently backcrossed with F344 females. With the speed congenic construction methodology, five rounds of successive backcrossing were performed to generate an F.ZF-*lepr* strain, which possesses leptin receptor null mutation in F344 genetic background. The F.ZF-*lepr* rat was intercrossed with F344-background congenic strains with OLETF-derived hyperglycemic QTL, namely F.O-*Nidd1/of* and F.O-*Nidd2/of*, to produce F.ZF/O-*lepr,Nidd1/of* and F.ZF/O-*lepr,Nidd2/of*. Male rats were then raised on standard laboratory diet until 15 weeks of age and phenotyped by OGTT (oral glucose tolerance test) assay.

**Results:** In OGTT, plasma glucose levels of neither F.O-*Nidd1/of* nor F.O-*Nidd2/of* rats at 15 weeks old were significantly different from those of the F344 rat (103.5 ± 5.3 mg/dl), suggesting that these loci are not sufficient when animals are relatively young. The F.ZF-*lepr* strain showed hyperglycemia at 30 min (179.5 ± 26 mg/dl) and at 120 min after glucose injection (169.0 ± 32 mg/dl). *Nidd1/of*, previously shown to influence more towards the late phase during OGTT, caused remarkably higher glucose levels at 120 min when combined with *lepr* mutation (264.2 ± 19 mg/dl, $p<0.01$). In contrast, *Nidd2/of* QTL, important for early response, showed elevated glucose levels at 30 min (319.5 ± 12 mg/dl, $p<0.01$). These data indicate that the both QTL exerted the effect presumably through obesity mediated by leptin activity deficiency.
**Conclusion:** Our congenic rats, which we propose as a model for "diabetes-prone" individual, are indeed normal when body weight is maintained at normal range. However, the single QTL, when stimulated with extreme obesity, results in severe plasma glucose deregulation. The enhanced phenotypic expression may help fine mapping of the causative genes. Furthermore, this study advocates the hypothesis that overt obesity is truly a high risk factor for type2 diabetes because it can lead to diabetes by interacting even with single QTLs.

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**Phenotypic and genetic analyses of subcongenic BB.SHR rat lines shorten the region on chromosome 4 bearing gene(s) for underlying facets of metabolic syndrome**

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**Background and aims:** Congenic BB.SHR (*D4Got41-Npy-Tacr1*; BB.4S) rats develop an incomplete metabolic syndrome with obesity, hyperleptinemia, and dyslipidemia as compared to their progenitor strain, the diabetes-prone BB rat. To narrow down the underlying gene(s), two subcongenic BB.SHR rat lines – briefly termed BB.4Sa and BB.4Sb – were generated.
**Materials and methods:** BB.4S rats were mated with BB, resulting rats were intercrossed, and appropriate homozygotes were selected and inbred. Male BB.4S (20), BB.4Sa (24) and BB.4Sb (26) were longitudinally characterized for facets of the metabolic syndrome up to an age of 32 weeks. To study the expression of genes located in the region of interest (prostaglandin F2 alpha receptor, *Ptgs2;* the eukaryotic translation initiation factor 2 alpha kinase 3, *Eif2ak3;* sialyltransferase 9, *Siat9;* methionine adenosyltransferase II, alpha, *Mat2a;* gelsolin-like capping protein, *Capg*), animals were killed, liver was removed, total RNA was extracted, transcribed into complementary DNA, and used for real-time PCR (ABIPrism7000).
**Results:** Body weight gain was comparable, serum triglycerides and leptin were significantly increased, and total cholesterol and HDL-cholesterol ratio were decreased in BB.4S compared to both subcongenics. Serum insulin was significantly higher in BB.4S and BB.4Sa than in BB.4Sb. The glucose tolerance demonstrated as glucose area under the curve (AUC) was significantly impaired after 28 weeks in both subcongenics, BB.4Sa and BB.4Sb, compared with BB.4S which suggests an impaired glucose tolerance with increasing age. The adiposity index showed a graduated decrease from BB.4S to BB.4Sb. Obvious differences in gene expression were only found in one gene, *Eif2ak3* also called *Perk* or *Pek*. The expression was significantly reduced in both subcongenics by about 70% compared to congenic BB.4S.
**Conclusions:** Based on the phenotype and genotype in BB.4S and its subcongenic derivatives, the most important region on chromosome 4 lies between *D4Got72* and *Tacr1*. *Eif2ak3* is mapped in this region. *Eif2ak3* regulates protein synthesis and is expressed in most tissues, with the highest expression in pancreatic islets and placenta. The role of *Eif2ak3* in maintaining the function of pancreatic β-cells has been corroborated by studies on knock-out mice lacking functional *Eif2ak3*. These knock-out mice are born with an apparently normal phenotype. Between 2 and 4 weeks of age, however, these mice gradually develop hyperglycemia, the protein synthesis is abnormally elevated and higher levels of endoplasmatic reticulum (ER) stress were observed. Considering the function of *Eif2ak3*, it may be a candidate gene for the development of glucose intolerance found in both subcongenics but not in BB.4S. Because subcongenic BB.4Sa and BB.4Sb are homozygous for BB and BB.4S for SHR alleles, the increase of AUC in subcongenics could be attributed to different *Eif2ak3* alleles in BB and SHR rats. This idea is supported by human studies where allelic variants were found in *Eif2ak3* leading to additional phenotypic features, including a predilection to severe hypoglycemic episodes, indicating hepatic impairment and renal failure. If allelic variants exist between BB and SHR, they could not only cause glucose intolerance but also a disturbed hepatic and renal metabolism.

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**Presenilin associated, rhomboid-like protein: a mitochondrial intramembrane protease associated with insulin resistance and Type 2 diabetes**

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**Background and aims:** Skeletal muscle insulin resistance plays a pivotal role in impaired whole body glucose disposal and contributes substantially to the development of type 2 diabetes. We therefore carried out large scale gene expression screening in skeletal muscle of *Psammomys obesus*, a unique polygenic animal model of obesity and type 2 diabetes, to identify differentially expressed mRNA transcripts potentially important in impaired glucose metabolism.
**Materials and methods:** cDNA obtained from skeletal muscle of lean, normal glucose tolerant and obese, type 2 diabetic *P. obesus* was hybridized to a Research Genetics Human Gene Filter (GF201) to identify differentially expressed transcripts. Taqman real time PCR in *P. obesus* and SNP typing in human subjects was utilised to investigate the role of differentially expressed genes in the development of type 2 diabetes.
**Results:** 54 genes with evidence of differential expression were mapped on the human genome. One gene, which was found to encode Presenilin associated, rhomboid-like protein (PSARL), mapped to chromosome 3q27 in a region likely to contain a diabetes susceptibility gene. In *P. obesus*, Taqman PCR showed that PSARL gene expression was reduced by ~50% in muscle of animals with diabetes ($p<0.05$), and was negatively correlated with plasma insulin ($r = -0.51$, $p<0.01$) and blood glucose concentrations ($r = -0.48$, $p<0.05$). Additionally, PSARL gene expression was increased by ~50% ($p=0.036$) in diabetic *P. obesus* exercise-trained for 3 weeks. To investigate the possible role of PSARL in human diabetes, we typed a SNP encoding a leucine to valine substitution in exon 7. This common polymorphism was found to have a strong effect on plasma insulin concentration ($p=0.0098$). Furthermore, there was evidence for substantial genotype-by-age interaction such that the importance of genetic variation in PSARL increased with age ($p=8.6 \times 10^{-5}$).
**Conclusion:** These data highlight a potential role for PSARL, a nuclear encoded mitochondrial rhomboid protease, in skeletal muscle insulin resistance, and thus may provide a new target for the treatment of type 2 diabetes.

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**The winged helix transcription factor FOXC2 C-512T gene polymorphism is associated with obesity and dyslipidemia**

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**Background and aims:** Over-expression of the human transcription factor FOXC2 gene (*FOXC2*) protects against insulin resistance in mice, and a common polymorphism (C-512T) has been suggested to be associated with insulin resistance in humans. Here we investigated the potential role for *FOXC2* as a candidate gene for type 2 diabetes, obesity and related phenotypes.
**Materials and methods:** A case-control study was performed in 390 type 2 diabetic patients (age 62.0 [54.8–67.9] years, BMI 28.9 [25.9–31.8] kg/m$^2$) and 307 control subjects (age 60.3 [53.4–67.2] years, BMI 26.4 [24.1–29.2] kg/m$^2$). The number of type 2 diabetics was increased to a total of 768 subjects for further studies of genotype-phenotype correlations. Two association studies in obese and normal weight non-diabetic subjects were performed. The first study consisted of 127 obese (age 39.0 [34.0–45.0] years, BMI 39.8 [35.8–44.5] kg/m$^2$) and 127 normal weight non-diabetic subjects matched for age and sex (age 40.0 [34.9–44.0] years, BMI 22.4 [21.0–23.7] kg/m$^2$). A replication study consisted of 223 obese (age 39.0 [34.0–45.0] years, BMI 39.0 [36.0–42.7] kg/m$^2$) and 231 non-obese subjects (age 37.0 [32.0–43.0] years, BMI 24.0 [22.0–25.4] kg/m$^2$). The *FOXC2* C-512T polymorphism was genotyped using PCR and cleavage with the restriction enzyme *Bbs* I.
**Results:** *FOXC2* C-512T allele and genotype distribution did not differ between patients with type 2 diabetes and control subjects, but the C/C genotype was associated with increased BMI (30.1 [28.0–33.3] *vs.* 28.6 [25.8–31.7] kg/m$^2$, $p = 0.01$) among type 2 diabetic patients and the T/T genotype with decreased waist circumference (88.0 [81.0–97.5] *vs.* 92.0 [84.0–100.5] cm, $p = 0.02$), and waist-to-hip ratio (0.86 [0.82–0.93] *vs.* 0.90 [0.82–0.96], $p = 0.03$) among control subjects. In the extended type 2 diabetic material, the C-allele ($p = 0.01$) and C/C and C/T genotypes ($p = 0.03$) were significantly over-represented in type 2 diabetic males with a concomitant diagnosis of the dysmetabolic syndrome. In the first association study for obesity, the C-allele showed significant association with obesity, odds ratio 1.74 (1.12–2.73; $p < 0.01$) for the C *vs.* T-allele and 1.81 (1.04–3.25; $p < 0.05$) for the C/C and C/T *vs.* T/T genotype. BMI was significantly higher in carriers of the C/C and C/T genotype in normal weight (22.7 [21.9–24.0] *vs.* 21.7 (20.7–23.3) kg/m$^2$, $p = 0.02$) but not in obese subjects (40.5 [35.2–46.3] *vs.* 41.3 [38.0–45.0], $p = 0.1$). In the replication study, the C-allele exhibited an increased risk for obesity under a recessive model, odds ratio 2.01 (1.15–3.52; $p = 0.01$). Obese carriers of the C-allele had significantly lower HDL-cholesterol (1.1 [0.9–1.3] *vs.* 1.2 [1.0–1.4] mmol/l, $p = 0.006$) and increased triglyceride levels (1.95 [1.30–2.68] *vs.* 1.60 [1.10–2.40] mmol/l, $p = 0.02$) compared to obese carriers of the T/T genotype.
**Conclusion:** *FOXC2* is associated with obesity, body composition and dyslipidemia but does not contribute to an increased risk for type 2 diabetes.

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**Screening, identification and association study of new single-nucleotide polymorphisms in candidate genes of diabetes Type 2**

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**Background and aims:** The metabolic syndrome is characterised by an impaired insulin secretion and action, adipositas, high blood-pressure and dyslipidemia. Although environmental factors play an important role in determining the risk of the disease, genetic variations contributes substantially to susceptibility. In addition, the lipid metabolism is also involved. Within the scope of the BMBF-Project *Dietary Fats and the Metabolism* we screened single nucleotide polymorphisms (SNPs) in genes involved in fat sensing (glucose-depending insulinotrophic polypeptide and its receptor), digestion (lipase, colipase), absorption and binding (fatty acid transporters, fatty acid- and acyl-CoA-binding-proteins), esterification (acyl-CoA-syntheses) and metabolism (AMP-Kinase).
**Materials and methods:** Subjects were taken from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study. Baseline examination was conducted between 1994 and 1998. During the first follow-up, 2.3 years after recruitment on average, 192 incident cases of type 2 diabetes were identified and medically proven by confirmation of the primary care physician. Cases were matched with 2 control subjects each by age and sex (n=384). The promotor, slice-site and exon regions of candidate genes were amplified by PCR from 47 DNA samples. To detect SNPs with a minor allele frequency of > 0.03, the obtained PCR products were sequenced using a cycle sequencing method and a high-throughput 96-capillary electrophoresis analyser (ABI PRISM 3700 DNA Analyzers).
**Results:** In the set of 22 genes, the lengths screened were 26.1 kb in promoter regions, 31.2 kb in intron/splice-site regions, 16.4 kb in 5´-/3´-untranslated regions and 23.3 kb in coding regions. The number of identified SNPs was 126, or a frequency of one SNP per 770 bp. Among the 126 SNPs, 86 (68%) are in non-coding regions and 40 (32%) in coding regions. More than 30% of identified SNPs are not found in public data bases or in the literature. At present, 13 SNPs were expected to be functionally significant are genotyped in a prospective nested case-control study of 192 incident cases of the type 2 diabetes and 384 sex- and age-matched control subjects taken from the EPIC-Potsdam study. The association tests were conducted with $\chi 2$ test and different logistic regression models. Nine SNPs in six different genes showed no statistical association with diabetes type 2. Evidence for association with disease status was found for a promoter variant of acyl-CoA-binding protein (RR=1.94, CI=1.0–3.6, p=0.045), a missense variant of ACBP (RR=2.9, CI=0.8–10.3, p=0.04), a promoter haplotype of intestinal fatty acid binding (RR=0.45, CI=0.24–0.87, p=0.017) and a missense variant of the pancreatic colipase (RR=0.349, CI=0.119–1.021, p=0.055).
**Conclusion:** Our study provide evidence that common variants in genes important for fat assimilation contribute to genetic susceptibility to diabetes type 2.
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**Role of sweet receptor gene in genetic susceptibility to Type 2 diabetes mellitus in Japanese subjects**

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**Background and aims:** Decreased sweet taste sensitivity has been reported in type 2 diabetic patients and diabetic animal models. The sweet receptor is known to be involved in the cephalic phase of insulin release. The contribution of the cephalic phase to overall insulin secretion in response to a glucose load is small, in the range of 1–3%. However, the cephalic phase has been suggested to be of considerable functional importance in regulating insulin secretion and glucose tolerance. Thus, diversity in sweet taste perception might be involved in type 2 diabetes development. The sweet receptor (TAS1R2) gene is localized to 1p36-p32, one of the regions possibly linked to type 2 diabetes in Japanese subjects. This study was designed to investigate the possible contribution of single nucleotide polymorphisms (SNPs) in TAS1R2 to type 2 diabetes development.
**Materials and methods:** We surveyed SNPs in the TAS1R2 gene in 388 Japanese subjects with type 2 diabetes mellitus and two control Japanese populations: one consisting of 176 elderly subjects who met stringent criteria for being non-diabetic, including age above 60 years and no evidence of diabetes (HbA1 c<5.6%), and another 240 subjects with normal glucose tolerance (NGT) on oral glucose tolerance test (OGTT). SNPs were identified by direct sequencing.
**Results:** We identified 10 SNPs with and four SNPs without amino acid substitutions in their coding regions. An amino acid substitution at position 317, from Arg (949C/950G: wild type) or Gly (949G/950G) to Ala (949G/950C), in the TAS1R2 protein was denoted as 317A. The allele frequency of 950C (317A) was significantly higher in type 2 diabetic patients than in both elderly normal (p=0.021) and NGT subjects (p=0.0001), whereas the allele frequency of other SNPs was essentially identical in these three groups.
Furthermore, in the NGT subjects, 950C (317A) was associated with a significantly lower insulinogenic index (the ratio of the insulin increment to that of plasma glucose 30 min after a glucose load), an indicator of the early insulin response on OGTT, indicating that early insulin secretion in response to a glucose load is reduced in subjects with 950C (317A)

(0.52 ± 0.26 vs. 1.07 ± 0.83, p=0.00004). Their insulin concentrations 1–2 hours after a glucose load were also significantly greater than those of subjects with the 950G allele. Thus, their area under the curve (ACU) insulin on OGTT was also significantly greater than that of subjects with the 950G allele (1332 ± 464 pmol/l vs. 1016 ± 422, p=0.02). The ISI composite, an insulin sensitivity index calculated from plasma glucose and insulin levels at OGTT, in the NGT subjects with the 950C allele, was also significantly lower than that of subjects with the 950G allele (2.98 ± 0.67 vs. 3.73 ± 0.76, p=0.0021).
**Conclusion:** These results demonstrate that the G950C (317A) polymorphism in the TAS1R2 gene is associated with a blunted early response and delayed enhancement of insulin secretion in response to an oral glucose load. Our results suggest that the sweet receptor has a significant role in regulating the cephalic phase of insulin release. This is the first report showing the TAS1R2 gene polymorphism to be associated with the development of type 2 diabetes in Japanese subjects.

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**A novel haplotype combination (111/121) in calpain-10 gene is significantly associated with Type 2 diabetes in Koreans**
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**Background and aims:** Genetic Variation in *CAPN 10*, the gene encoding the cysteine protease calpan-10, has been associated with type 2 diabetes mellitus (T2 DM) in Mexican-Americans and two northern-Europeans (Finland and Germany). Nevertheless the other populations including Japanese showed variation of *CAPN 10* does not strongly associated with T2 DM.
**Materials and methods:** We replicated to genotype 3 SNPs in *CAPN 10* defined a diabetes high-risk haplotype in Mexican Americans: UCSNP-43, -19, and 63. A total of 454 Korean patients with T2 DM (M/F, 230/222; age, 53.3 ± 11.0 yr; BMI 24.4 ± 3.5; HbA1 c, 8.4 ± 3.3) and a total of 236 non-diabetic Koreans (M/F, 124/112; age, 62.6 ± 5.1 yr; BMI 23.8 ± 2.7) with no family history of diabetes. The genomic DNA was isolated and 3 SNPs were examined by the electrophoresis of the PCR product by size or after digested with enzymes (*Nsi* I and *Hha* I).
**Results:** There was no significant association with diabetes in 3 SNPs: UCSNP-43 (allele 1, G; 2, A; p=0.402), UCSNP-19 (allele 1, 2 repeat of 32 bp; allele 2, 3 repeat; p=0.153; in genotypic distribution, $\chi^2$=3.37, p=0.07), and UCSNP-63 (allele 1, C; 2, T; p=0.326). The haplotype allelic distributions with PHASE program were 8 alleles (121, 122, 111, 112, 221, and 3 rare alleles) and showed significant association with T2 DM ($\chi^2$=18.78, p=0.001). After adjusted with sex and age, haplotype-allele 111 showed high risk of T2 DM (OR=2.23, p=0.001). And in the haplotype combinations, the haplotypes, 111/121 and 112/112, showed high risk of T2 DM (OR=3.34, p=0.001, and OR=2.38, p=0.049 respectively). The high-risk haplotype (112/121) in Mexican Americans was not significant (p=0.815). And especially in the obese subgroup (BMI >24.4), the haplotype 111/121 showed more increased risk of T2 DM (OR=4.29, p=0.004), comparing with less obese (BMI < 24.4, OR=2.94, p=0.005).
**Conclusion:** We investigated to genotype 3 well-known SNPs of *CAPN 10* in Korean T2 DM and found a novel high-risk haplotype (111/121) in our population. The risk of T2 DM was more increased in obese subgroup suggesting insulin resistance.

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**Large-scale association studies of candidate genes and their interactions in Type 2 diabetes**
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**Background and aims:** Large-scale studies provide the most effective way of establishing whether or not a given candidate variant truly influences susceptibility to T2D. Following successful application of this approach to establish the role of variants in genes influencing beta-cell function (*KCNJ11*, *CAPN10*), here we report analyses of several single nucleotide polymorhpisms (SNPs) implicated in insulin action [PPARG/P12A (rs1801282); TNF/G-308A (rs1800629); TNF/G-238A (rs361525); IRS1/P512A (rs1801276); IRS1/R971G (rs1801278)]. As these molecules interact at the functional level, it is plausible that their genetic variants may also act in concert to increase risk of developing T2D. The aim of this study has been to investigate the presence of epistatic effects between PPARG, TNF and IRS-1 genetic variation.
**Materials and methods:** All SNPs were genotyped (Pyrosequencing or MASSARRAY) in >2500 subjects including 973 T2D cases (565 Warren 2 probands; 249 young-onset T2D and 159 trios) and 1257 control subjects (347 population cases; 910 birth-cohort parents).
**Results:** The IRS-1 and TNF -308 SNPs were not associated with T2D in this dataset. Carriage of the TNF -238*A allele was associated with an increased risk of the disease (OR: 1.35, 95% CIs: 1.06–1.70, p=0.01). At PPARG, we found strong support for previous data indicating that homozygosity of the proline variant at codon 12 (rs1801282) increases T2D risk (OR: 1.39, 95% CIs: 1.12–1.73, p=0.002). When logistic regression was used to model case/control status on PPARG, TNF and IRS-1 genotypes, no significant pairwise interactions among the loci were identified. Although this has been the largest study to date, sufficient power to detect epistatic effects between any of the T2D susceptibility genes was not achieved. Power calculations indicated that a 15-fold increase in sample size would be necessary in order to detect a significant interaction between the PPARG and TNF SNPs examined.
**Conclusion:** Gene-gene interactions of modest effect sizes are likely to underlie the aetiology of complex traits, thereby emphasising the need for large-scale, collaborative efforts in order to unequivocally address their presence.

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**Mutations in the glucokinase gene: genotype / phenotype correlation**
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**Background and aims:** In the beta cells, Glucokinase (GCK) acts as the `glucose sensor` that normaly couples insulin secretion. Mutations in the GCK gene were the first genetic defects implicated in an inherited predisposition to diabetes. The aims of our study were to investigate the prevalence of mutations G264S abd IVS8+2T → C in the GCK gene, in family members of two patients with congenital diabetes attributed to these mutations and to describe the relationship between clinical characteristics and the presence of these mutations.
**Materials and methods:** Ninety family members were genotyped for the G264S and IVS8+2T→C mutations in the GCK gene. Clinical, laboratory and historical data were collected via chart review (blinded to genotype results).
**Results:** A total of 90 individuals, of which 81 were studied for the IVS8+2T→C mutation, were investigated. Thirty one (38%) IVS8+2T→C heterozygotes were identified. Of these, 25/31 (80.6%) have diabetes or IFG, compared with 3/50 (6.25%) of non-carriers. Mean BMI for heterozygotes who have diabetes was 24.6, for non-diabetes heterozygotes 21 and for non-carriers 23.7. Mean HbA1 c for heterozygotes with diabetes was 6.4%, for heterozygotes with normal glucose 5.63% and for non-carriers 5.13%; Twelve subjects were investigated for the G264S mutation, 3/12 (12.5%) were carriers, one has diabetes and 2 have IFG, their mean BMI was 26.82 and mean HA1 c 5.63%. Nine of 12 (75%) were non-carriers, all have normal glucose levels, their mean BMI was 30.83, and mean HA1 c 4.74%.
**Conclusion:** These data support the association between the GCK gene mutations G264S and IVS+2T→C and the development of diabetes. Moreover, in subjects with IVS8+2T→C mutation there was a clear correlation underline the importance of prevention of diabetes in heterozygous subjects.
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**Identification of early-onset diabetes due to a frame-shift mutation in Ipf-1 gene in Japanese**
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**Background and aims:** Ipf-1 (or PDX-1) is a transcriptional factor with a homeobox domain, and plays a crucial role both in the early developmental process of pancreas and the transcriptional regulation of insulin and other genes in mature beta cells. Pdx1 gene knockout mice, as well as human null homozygotes, exhibit pancreas agenesis. Heterozygous mutation in Ipf-1 gene is reported to cause early-onset diabetes, called MODY4, but the reported cases are extremely rare, and no Japanese patients have been found so far who have diabetes caused by Ipf-1 gene. Therefore, we investigate the role of Ipf-1 mutations in Japanese diabetic subjects.
**Materials and methods:** We screened 181 Japanese diabetic patients for nucleotide changes in the whole coding region of Ipf-1 gene. For the one mutation identified, we performed functional analysis of the mutant protein in vitro as follows: 1) the DNA binding to A3 region of human insulin promoter by electrophoresis gel mobility shift assay (EMSA), 2) subcellular localization of Ipf-1 protein fused with GFP (green fluorescent protein), and 3) the transcriptional activity in COS-1 cells using human insulin promoter with luciferase as reporter gene.
**Results:** One insertional mutation was found in a patient whose onset of diabetes is the age of 24. He is a heterozygote, and the mutant allele has a single adenine insertion at the position of nucleotide 746 of Ipf-1, which causes frameshift and gives rise to a truncated protein. The mutation is novel and has not been reported before. The family study revealed that his diabetic mother is also a heterozygote, but the mutation was not found in the other patients or in 210 non-diabetic Japanese controls, suggesting that it is a rare mutation rather than polymorphism.Functional analysis revealed that the mutant Ipf-1 protein binds to target DNA, and normally localized within nucleus, consistent with the fact that the predicted mutant still contains the DNA binding domain and the nuclear localization signal (NLS). However, the transcriptional activity on insulin promoter was significantly decreased to 60% of wild-type protein. These results demonstrated that this is a loss-of-function mutation in Ipf-1 gene
**Conclusion:** We identified a novel frameshift mutation in Ipf-1 gene in a Japanese diabetic patient. Together with clinical phenotype, it is strongly suggested that this mutation is responsible for the pathogenesis of his young-onset diabetes and can possibly be considered as MODY4. This may raise the possibilities that mild impairment of Ipf-1 function can contribute to the development of human diabetes mellitus.
*Supported by: the Program of Fundamental Studies in Health Sciences of Pharmaceuticals and Medical Devices Agency.*

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### Molecular analysis of HNF-4α (MODY1), GCK (MODY2) and HNF-1α (MODY3) in 77 Spanish families with MODY

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**Background and aims:** Maturity Onset Diabetes of the Young (MODY) is a monogenetic type of diabetes mellitus characterised by early onset, usually before 25 years of age, an autosomal dominant mode of inheritance and absence of pancreatic autoinmunity markers. Mutations in six genes have been currently shown to cause most of the MODY cases, but in almost all studies around a 60% of the cases have been explained by alterations in just three of these genes. All of them encode proteins involved in the glucose homeostasis of pancreatic β-cells: two nuclear transcription factors; hepatocyte nuclear factor-4α (*HNF-4α* /MODY1) and hepatocyte nuclear factor-1α (*HNF-1α* /MODY3) and the enzyme of the glycolitic pathway glucokinase (*GCK*/MODY2). The aim of the present study is to report the prevalence and the clinical characteristics of MODY1, MODY2 and MODY3 gene mutations in our population.
**Materials and methods:** Molecular analysis of *HNF-4α*, *GCK* and *HNF-1α* was performed in unrelated probands of a total of 77 families with clinical diagnosis of MODY. All exons and the promoter regions of the *HNF-4α* and *HNF-1α* genes were analysed using direct sequencing, while exons of the *GCK* gene were first screened for alterations using SSCP (single-strand conformational polymorphisms). Then, those exons with abnormal migration pattern were sequenced to define the mutation.
**Results:** Mutations in the *GCK* gene were identified in 53 (68.8%) of the 77 families studied. Out of these, we describe 38 different mutations, 11 of which have been previously reported and 27 are novel mutations. The analysis of *HNF-1α* was performed in the remaining 24 families without mutation in *GCK*. We found that 8 families (10.4%) had a mutation in this gene and we identified 6 different alterations, one has not been described before, while the rest have been previously associated with MODY. To date, *HNF-4α* gene has been analysed in 12 out of the 16 families without changes in none of the two genes previously studied. No mutation has been found.
**Conclusion:** 1) 79.2% of MODY cases in our population are due to mutations in *GCK* (MODY2) or *HNF-1α* (MODY3) genes. 2) No mutations in *HNF-4α* (MODY1) have been found in our population. 3) We describe 27 novel mutations in the *GCK* gene and 1 in the *HNF-1α* gene.
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### Patient-derived mitochondrial DNA mutation A3243G impairs glucose metabolism in cybrid cells

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**Background and aims:** Mutations in the mitochondrial DNA (mtDNA) cause rare but debilitating human diseases, including mitochondrial inherited diabetes and deafness (MIDD). Such diseases are often associated with mtDNA A3243G point mutation on the tRNA (Leu) gene. Cells, obtained from the parental human cell line 143B and depleted of mtDNA (ρ°), were used as recipients for mtDNA from a patient with A3243G mutation. We studied the impact of the mutation on mitochondrial metabolism when comparing stimulation of these cybrid cells by glucose versus the mitochondrial substrate pyruvate.
**Materials and methods:** We used clonal cybrid cell lines prepared with ρ° cells containing patient-derived either mutant (M12) or wild type (W20) mtDNA following mitochondria-mediated transformation. We compared metabolic activation using glucose or pyruvate by measuring mitochondrial membrane potential (rhodamine-123 fluorescence) and cellular ATP levels in cell extracts or in living cells (luciferase-mediated photon emission).
**Results:** In 143B and W20 cells the protonophore FCCP could dissipate, and therefore revealed, established mitochondrial membrane potential (ΔΨm). Conversely, in ρ° and M12 cells resting ΔΨm was negligible. Upon certain conditions, some ΔΨm could be generated in ρ° and M12 cells, attributed to reverse activities of ATP/ADP translocase and ATP synthase, in this conditions consuming glycolysis-derived ATP. Accordingly, some ΔΨm in ρ° and M12 cells was generated by glucose (5 mM) but not by the mitochondrial substrate pyruvate (1 mM), whereas both nutrients strongly increased ΔΨm in control 143B and W20 cells. Basal ATP contents in ρ° and M12 cells were 222 and 100 times lower than in 143B or W20 cells, respectively; i.e. 0.09 ± 0.04 (ρ°), 0.17 ± 0.08 (M12), 20.0 ± 4.2 (143B), and 17.2 ± 4.0 (W20) nmols ATP/mg protein. Upon glucose (5 mM) stimulation ATP contents of these cells were: 9.7 ± 1.6 (ρ°), 12.5 ± 2.0 (M12), 24.0 ± 5.1 (143B) and 20.7 ± 5.8 (W20) nmols ATP/mg protein. Therefore, the following fold increases in cytosolic ATP levels were: 1.2-fold in 143B, 1.2-fold in W20, 108-fold in ρ°, and 74-fold in M12 cells, $p < 0.05$. In order to evaluate the kinetics of cytosolic ATP concentrations, we next monitored cybrid cells expressing luciferase. Glucose (5 mM) stimulation resulted in sustained elevation of cytosolic ATP levels in 143B and W20 cells, whereas ATP increases were only transient in ρ° and M12 cells. Pyruvate (1 mM) augmented cytosolic ATP levels in control 143B and W20 cells, but not in ρ° and M12 cells.
**Conclusion:** Expressed in the cybrid cell model, the mtDNA A3243G mutation impaired mitochondrial membrane potential and mitochondrial ATP generation. The initial ATP content of ρ° and M12 cells was much lower when compared to control 143B and W20 cells. Upon glucose stimulation, ρ° and M12 cellular ATP contents were markedly increased, but only approaching basal ATP levels of 143B and W20 cells. Mitochondrial substrate pyruvate was able neither to build sustained ΔΨm nor to increase ATP levels in mutant cells (M12). Therefore, mitochondrial defects provoked by mtDNA A3243G mutation might impair beta cell function, consequently contributing to the diabetic phenotype in MIDD.
*This work was supported by the Leenaards and Swiss National Science Foundations*

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### Mitochondrial DNA phylogenic lineages for mapping Type 1 diabetes genes

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**Background and aims:** Mapping type 1 diabetes (T1D) genes requires collection of large population samples, which results in accumulation of genetic heterogeneity. Paradoxically, gene effects become even more difficult to detect due to increased variation in linkage disequilibrium (LD) pattern and possible locus/allele heterogeneity. To obtain a genetically homogeneous patient series with uniform LD pattern, identification of related patients/families could be an approach. We defined maternal genealogical lineages of T1D patients and analyzed linkage and association at genomic disease susceptibility loci in the family clusters identified. The mtDNA diversity of T1D patients and that of the general population was also compared.
**Materials and methods:** MtDNA Hypervariable Region I was sequenced and nine mtDNA haplogroup defining polymorphisms were analysed in 80 Finnish affected sibpair (ASP) families, in 92 nuclear families and in 152 unselected newborn infants, – all from Northern Finland. Phylogenic trees were constructed by Phylip 3.5 (NJ algorithm). HLA DR-DQ, IDDM2 (2 markers), CTLA4 (3 markers), IDDM9 (17 markers) and chromosome X (29 markers) were analysed in the various mtDNA lineages. TDTPHASE and Genehunter 2.0 were used for association and linkage studies. LD, analysis of molecular variance (AMOVA) and gene diversity was calculated using Arlequin.
**Results:**Phylogenetic analysis showed a markedly lower mtDNA diversity among patients when compared to controls. The mean number of pairwise differences between mtDNA haplotypes was lower among the patients affected by T1D (3.98 ± 2.01) than among the controls (5.39 ± 2.60). AMOVA indicated that 0.6% of the total mtDNA molecular variation was explained by differences between cases and controls (Fst 0.00571) and pairwise comparison of population samples confirmed a significant difference (corrected PXY p value <10–4). A comparison of HLA haplotypes among patients with various mtDNA haplogroups revealed that maternal DR3 and DR4 risk haplotypes were more common in haplogroup H than in other haplogroups (80.0% vs. 59%, p=0.02). Among the maternally transmitted alleles of the IDDM2-insulin gene region -2221 MspI polymorphism the protective T allele was more rare in the mtDNA haplogroup H than in the random series of T1D patients (2% vs. 9%. p=0.035). Variation in the transmission of markers was detected at IDDM2 (-2221 MspI, HUM-TH01), and at the IDDM9 region (D3S1303, D3S2302, D3S3552, D3S1269, D3S1589) in family sets stratified according to their mtDNA haplogroups. The ASP family set with haplogroup H showed a multipoint lod score of 1.8 at DXS8076, while no linkage was detected in the total series. Variation in LD at the CTLA4 gene region was seen between three distinct mtDNA lineages of patients that ranged from weak to complete LD (+49 A/G - ATn D′ = 0.37, 0.58 and 1.00).
**Conclusion:** T1D patients are less diverse at the mtDNA level than the general population. Patients who belong to distinct mtDNA lineages display different LD patterns and variation in disease association and linkage at genomic T1D loci.
*We thank the Juvenile Diabetes Research Foundation and the European Foundation for the Study of Diabetes for their support.*

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# Metabolic syndrome and prediction of Type 2 diabetes

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### Metabolic syndrome and antioxidative activity in early glycoregulation disorders

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**Background and aims:** Early glycoregulation disorders, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are associated with metabolyc syndrome (MS). All these are important risk factors for diabetes mellitus type 2 (DM type 2), atherosclerosis and cardiovascular disease. The aim of this study is: (a) to analyze parameters of insulin sensitivity and secretion, glycoregulation, lipid status, parameters of antioxidant activity, microalbuminuria (MI) and homocysteine (HCY) in patients with obesity (I), IFG (II), IGT (III) and newly diagnosed DM type 2 (IV) patients; (b) to determine the percentage of metabolic syndrome at these patients.
**Materials and methods:** The study included 148 obese individuals (BMI >25 kg/m$^2$) over 45 years. The oral glucose tolerance test (OGTT)- WHO, WG, 1998. revealed obesity in 23.6%, IFG in 12.2%, IGT in 35.8%, diabetes mellitus type 2 (DM2) in 28.4% of patients. Insulin sensitivity was determined by the insulin/glycemia (I/G) ratio and HOMA IR, insulin secretion by HOMA b; lipid status was determined by spectrophotometry (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides) and immunochemical method (Apo A1, Apo B, Lp(a)1. We examined erythrocyte dismutase (E-SOD), erythrocyte glutation peroxide (E-GPX) and total antioxidant status (TAS) as markers of antioxidant defense. MI was determined by nephelometric method.
**Results:** The metabolic syndrome accounted for 38.2% in obese patients, while it was almost double in other groups (IFG - 72.2%, IGT - 76.0%, DM2 - 70.7%). BMI indicated extreme obesity in all groups. HOMA IR (3.1 ± 1.6; 3.5 ± 2.2; 5.6 ± 3.7; 6.6 ± 4.0) was elevated in IGT and DM2 groups, while HOMA β (219.3 ± 110.5; 105.3 ± 54.8; 225.2 ± 323; 0.146.3 ± 213.3) showed statistical significance (p<0.05) between obese and IFG and between obese and DM2. LDL/HDL ratio and Apo Al/ApoB ratio were at the upper maximum level and did not differ statistically between groups. Lp(a) was slightly increased in IGT patients and considerably increased in IFG ones. Blood pressure was moderately increased in all groups of patients with no statistical difference between groups. All antioxidant protection markers were dicreased (SOD:846.7 ± 112.2; 824.0 ± 93.5; 838.0 ±l0l.3; 849.2 ± 87.6; GPX: 25.4 ± 7.7; 23.8 ± 5.1; 23.1 ± 4.8; 23.9 ± 4.3;TAS: 1.28 ± 0.2; 1.27 ± 0.2; 1.28 ± 0.2; 1.26 ± 0.3), homocystein was at upper maximum level (II: 11.7 ± 3.0; III: 12.3 ± 4.6; IV: 11.5 ± 2.7), and there was no important statistical difference between groups. MI showed a statistical importance (p<0.05) at obese patients with MS compared to those without MS.
**Conclusion:** Results obtained indicate that a metabolic syndrome exists at two thirds of patients with early glycoregulation disorders (IFG, IGT) and newly diagnosed diabetes mellitus type 2, and that it is associated with increased insulin resistance, hypertension, hyperlipoproteinemia and reduced antioxidant protection. This contributes to endothelial disfunction and accelerated atherosclerosis.

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### New definition of IFG: impacts on metabolic syndrome definition and prediction of diabetes

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**Background and aims:** American Diabetes Association has recently lowered the IFG definition level to 100 mg/dL instead of the previous 110. The purpose of this study was to evaluate if this change should affect definition of the metabolic syndrome and prediction of diabetes incidence.
**Materials and methods:** In Tehran Lipid and Glucose Study, 3995 people above the age of 20 (2353 female), were followed up for 1 to 4 years till the end of 2003. In this cohort study, metabolic syndrome at the beginning of the follow-up was defined, according to NCEP criteria, by the presence of three or more of the following components: abdominal obesity,

hypertriglyceridemia, low HDL-C, high blood pressure, and high fasting glucose (above 110 mg/dL). The proposed definition of the syndrome was the same except for replacing the high FPG level with the new 100 mg/dL cut-off. Diabetes was defined as a fasting blood glucose above 126 or 2-hour post glucose load above 200 mg/dL, or use of hypoglycemic medication. ROC analysis and logistic regression (adjusted for age and sex) were used to compare the ability of the two definitions of metabolic syndrome and IFG alone in predicting incident diabetes in previously non-diabetic individuals.
**Results:** Mean follow-up duration was 3.1 years. During this period, 8% of those with the old definition and 8.3% with the newly proposed definition of metabolic syndrome developed diabetes. The odds ratio for incident diabetes in individuals with the old definition of metabolic syndrome was 3.1 (CI95%: 2.1–4.6) which increased to 4.2 (CI95%: 2.8–6.3) by the new definition. By the old definition, metabolic syndrome was 58.1% sensitive and 72.4% specific in predicting diabetes. Positive predictive value was 8% and negative predictive value 97.7%. The area under the ROC curve was 0.70 (95%CI: 0.650.75). By the new definition, the sensitivity increased to 67.5% while the specificity decreased to 69.8%. Both positive and negative predictive values increased slightly under the new definition (8.3% and 98.2%). The area under the ROC curve was almost the same as the old definition: 0.73 (95% CI: 0.69–0.78). When considering IFG alone, the old definition was 27.4% sensitive and 97.3% specific in predicting incident diabetes (Positive predictive value:29.1%, negative predictive value: 97.1%). The new definition had a higher sensitivity (63.2%) and a lower specificity (85.9%). Positive and negative predictive values of the new definition were 15.4% and 98.3%, respectively. The odds ratio for incident diabetes with the old IFG definition alone was 12 (95% CI: 7.5–19.3), which decreased to 9.5 (95% CI: 6.4–14.2) in the new definition.
**Conclusion:** A new definition of the metabolic syndrome, using the new cut-off for IFG recommended by ADA is suitable since it can predict incident diabetes better than the current definition.

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**Impaired fasting glucose as a predictor of future diabetes**
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**Background and aims:** The ADA recently lowered the diagnostic criteria for impaired fasting glucose (IFG) to 100 mg/dl. This revision will label a substantial proportion of the population as IFG. There is no doubt that these glucose values define a group with an increased risk of developing diabetes in the near future. This study aimed to estimate the risk of developing diabetes in people identified as IFG in Israel, according to the new criteria, using blood glucose records of a large health service in Jerusalem.
**Materials and methods:** We reviewed all glucose samples performed between Jan.–Jun.2001 from a central database of a health service in Jerusalem. All blood glucose values between 100–110 mg% were recorded. If a patient had several glucose measurements, the highest was taken. We eliminated patients with records of anti-diabetic drugs and patients who had HbA1C measurement above 5.8% during Jan.–Dec.2001. We followed this group of patients with the following data during Jan.–Dec.2003: highest blood glucose, highest HbA1C (if performed) and anti diabetic drugs. We eliminated patients with no recorded glucose levels during 2003. Patients were considered to develop diabetes if during 2003 they had either blood glucose measurement equal to or above126 mg% or their HbA1C >6.5% or if they were treated with anti -diabetic drugs.
**Results:** 10,582 people with blood samples of 100–110 mg% were recorded during the first half of 2001. 1,240 patients were eliminated due to receiving medical treatment for diabetes or HbA1C above 5.8%. Repeated blood glucose measurements during 2003 were missing in 3,222 patients. The remaining 6,119 made up the sample group to be followed during 2003. In 2003, 739 had either glucose above 125 mg%, HbA1 c above 6.5% or received anti-diabetic medication. Thus, the probability to develop diabetes by these criteria was 12% in 3 years. The risk of developing diabetes according to the same criteria as above, was 9.1% if blood glucose was 100–105, and 16.7% if glucose was 106–110 in 2001.
**Conclusions:** This retrospective analysis shows an extremely high percentage of progression from IFG to diabetes within 3 years. The progression was in linear correlation with the level of blood glucose during 2001. This high percentage of diabetes might be partly explained by a selected high risk group that performed frequent blood glucose measurements and thus were more frequently included in the sample. Some unrecognized diabetics were probably included in the 2001 sample. Appearance of diabetes reflects the natural progression of the disease in these people. Yet, for this „higher risk" group, blood glucose level above 100 mg% is a strong predictor of future diabetes.

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**Impaired fasting glucose has advantages in predicting Type 2 diabetes compared to impaired glucose tolerance when combined with BMI and HbA1c: a Swedish population based incident case-referent study**
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**Background and aims:** Type 2 diabetes mellitus (T2DM) is increasingly considered a preventable disease. There is a need of simple methods to identify individuals at risk for development of T2DM. The aim of this study was to test if impaired fasting glucose (IFG), in combination with glycated haemoglobin (HbA1 c) and body mass index (BMI), is as effective as impaired glucose tolerance (IGT) in identifying high risk subjects for future T2DM.
**Materials and methods:** A population based incident case-referent study was performed in Umeå municipality (pop135 000) within the Västerbotten Intervention Program. At the age of 40, 50 and 60 all inhabitants are invited to a health survey in primary care. An OGTT is performed, BMI is calculated and fasting blood samples are drawn and stored at –80° until analysis. Subjects with diagnosed T2DM in the area were identified by assessing all computerized patient records of the Dept of Medicine at the regional hospital and of all primary care centers (ascertainment > 95%). Prevalence in the population was estimated to 5.2%. Data from all 33 336 participants in the health survey 1989–2000, and data from all 6088 subjects with T2DM were merged, and 237 cases were identified. Cases were free from diabetes at the time of the health survey, but developed T2DM after a mean time of 5.4 years. Two referent subjects who had not developed clinical T2DM were selected for each case, matched for gender, age and year of health survey.The analysis compares two models with three risk criteria: IFG or IGT both used together with BMI 27 and HbA1 c 4.7 (normal range 3.6–5.3). HbA1 c of 4.7 is the 90$^{th}$ percentile in the referent distribution. Proceeding from odds ratios and based on Rothman´s definition of interaction and the relative risk due to interaction, the proportion of etiological cases attributable to a single criterion or combinations of criteria were calculated
**Results:** Combinations of two or all three criteria explained 48% of the cases in the IFG-model and 43% in the IGT-model, the sensitivity was 0.61 and 0.58 respectively, the specificity was 0.91 and 0.92 respectively. The positive predictive value was 0.27 and 0.28 respectively and the negative predictive value was 0.98 in both models.
**Conclusion:** Individuals with combinations of the risk criteria in either model are at high-risk for development of T2DM. The IFG-model performed at least equally well as the IGT-model and has the advantage of being inexpensive and easy to perform in everyday clinical practise.

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**Impaired fasting glycaemia in 743 French men: five years outcome and predictive factors**
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**Background and aims:** The progression to diabetes of men with impaired fasting glucose (IFG) (fasting plasma glucose (FPG): [6.1–6.9 mmol/l]) is poorly described. IFG is either a transient abnormality with a return to normal FPG or a first step leading to type 2 diabetes if risk factors are not promptly corrected. Therefore, we reassessed five years later men initially classified as IFG who were volunteers for a free medical check-up. We aimed at identifying predictive factors comparing the initial characteristics of men who became diabetics to those who returned to normal FPG five years later.
**Materials and methods:** 9,314 men, aged 20–60 years, noted their drug consumption and a nurse estimated their smoking and physical activity. Nutritional profile was assessed by an 18-item self-administered questionnaire which detailed quantities and frequencies of usual intakes. Fasting blood samples were collected. A physician asked for personal and family medical history, measured supine blood pressure at rest, height, weight and girth and gave advice if necessary. Same data were collected first when IFG

was stated and 5 years later. Odd-ratios were calculated for each data and adjustment for age and BMI used generalised linear models.
**Results:** IFG was found in 743 men (8%) at the first medical check-up. Among these subjects, 17% became diabetics five years later, 44% returned to normal FPG and 39% remained classified as IFG. The odd-ratios of developing diabetes was 4.5 (95% CI 1.7–10.2) for men with a familial history of diabetes, 3.4 (2.0–5.5) if BMI was ≥ 25 kg/m$^2$, 2.9 (1.9–4.4) if girth was above 89 cm, 2.8 (1.8–4.2) if triglyceride levels ≥ 2 mmol/l and 1.9 (1.2–2.8) for men having poor eating habits. Several factors were still significant after adjustment on age and BMI between men who returned to normal FPG and those who became diabetics: familial history of diabetes ($p < 0.0001$), triglyceride levels ($p < 0.00003$), and to a lesser extent haemoglobin ($p=0.04$) and haematocrit ($p=0.03$). Similarly, active tobacco consumption ($p = 0.01$), irregular physical activity ($p = 0.05$) or unbalanced eating behaviour ($p = 0.005$) were still significative. Girth, systolic and diastolic blood pressure, cholesterol, GGT and creatinine clearance were only significant before adjustment. In spite of targeted advice, subjects who became diabetics corrected less their lack of physical activity or daily breakfast than the subjects returning to normal FPG.
**Conclusion:** In these French men, 17% of subjects with IFG became diabetics five years later. Overweight or android obesity, familial history of diabetes, high triglyceride levels and to a lesser degree active tobacco use and unbalanced eating habits were the main predictive factors of diabetes. These findings are important for planning behavioural or drug strategies as soon as subjects have IFG in order to try to prevent the occurrence of diabetes.
*Supported by: Laboratoires Lilly, Aventis*

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**Low first phase insulin response predicts the onset of Type 2 diabetes in an IGT population: the HOORN-IGT Study**
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**Background and aims:** Beta-cell dysfunction and insulin resistance are predictive for incident diabetes. The disposition index expresses the capacity of the beta-cell to compensate for decreased insulin sensitivity. The hyperglycaemic clamp provides the gold standard measure of beta-cell function, i.e. first phase insulin secretion, and a measure of insulin sensitivity, the M/I ratio. The aim of this study was to analyse whether beta-cell dysfunction or insulin resistance is the strongest predictor for incident diabetes in persons with IGT.
**Materials and methods:** Random sample of 50–75 year-old persons from the city of Hoorn. Persons with a FPG >5.5 mmol/l underwent an OGTT, repeated within two weeks if the 2 hr PG exceeded 7.8 mmol/l. Persons with a mean 2 hr PG between 7.8 and 11.1 mmol/l (n=109) were selected. All persons underwent a hyperglycaemic clamp. During 8 years, an annual FPG was determined, followed by an OGTT if FPG > 7.0 mmol/l to assess incident diabetes.
**Results:** The cumulative incidences according to WHO`85 and WHO `98 criteria were 22% and 30.5%, respectively. In the Cox-regression analysis the absence of a first insulin secretion phase was a strong predictor for incident diabetes. The disposition index was only predictive for the WHO'85 criteria (table). Even corrected for M/I ratio the first insulin secretion phase kept its strong predictive value.
**Conclusion:** Impaired first phase insulin secretion was most predictive for incident diabetes in persons with IGT. The disposition index, expressing the compensatory capacity of the beta-cell for insulin resistance was predictive of incident DM [WHO '85] These results show that in particular impaired beta-cell function, and not insulin resistance, predict incident DM in IGT.

Cox regression analysis, all adjusted for sex and age

| Cox regression models<br>All adjusted for age and sex | WHO '85<br>Relative Risk<br>(95% CI) | WHO '98<br>Relative Risk<br>(95% CI) |
|---|---|---|
| Model 1: impaired 1st insulin secretion phase | 8.1 (2.86–22.4) ** | 4.5 (2.18–22.4)** |
| M/I ratio | 0.94 (0.87–1.03) | 0.99 (0.95–1.05) |
| Model 2: disposition index:<br>tertile 3 vs tertile 1 | 8.99 (2.01–40,21)* | 2.19 (0.89–5.34) |

*Supported by: Dutch Diabetes Foundation*

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**8 year follow up of women with previous gestational diabetes**
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**Background and aims:** Gestational diabetes mellitus strongly increases the risk to develop permanent Diabetes mellitus years after delivery. We hereby report the 8 year follow up of 317 women with previous gestational diabetes mellitus (GDM).
**Materials and methods:** 317 women with GDM were recruited in several centres in Germany between 1989 and 1996 and treated during pregnancy with insulin (38%) or diet (62%). BMI, duration of pregnancy, age at delivery and further treatment were recorded. Serum of the patients was screened for the presence of islet antibodies to GAD and IA-2. During follow-ups at 6 month, 3, 5 and 8 years, patients underwent a physical examination by their general practitioner and an oral glucose tolerance test for detection of postpartal diabetes. High sensitive C-reactive protein (hsCRP) was measured in the first postpartal serum sample that was taken between 6–9 month after delivery. Body mass index (BMI), insulin requirement during pregnancy (White-class), age at delivery, duration of pregnancy, presence of islet antibodies and hsCRP were evaluated as risk factors for the progression to postpartal diabetes mellitus.
**Results:** Of 317 women, 27 (8.5%) patients were positive for islet antibodies. Of these 27 patients, 23 (85%) progressed to type 1 diabetes within the first year post partum and all 27 patients had developed type 1 diabetes by the end of the observation period. Insulin treatment during pregnancy, BMI, age at delivery, duration of pregnancy or hsCRP did not significantly affect the rate of progression to diabetes in antibody positive patients.
During the observation period, 115 (40%) of antibody negative patients progressed to type 2 diabetes with a median manifestation time of 376 days. Insulin treatment during pregnancy, BMI and hsCRP-levels were predictive of postpartal type 2 diabetes mellitus by univariate analysis ($p<0.0001$, $p<0.0001$ and $p=0.013$, respectively). hsCRP levels correlated with BMI and these were dependent risk faktors. By multivariate analysis, both insulin treatment during pregnancy (White class) and BMI remained independent risk factors ($p<0.0001$ for both) while hsCRP did not further improve the risk model. In contrast to previous studies, age at delivery and duration of pregnancy did not influence the diabetes risk.
**Conclusion:** Patients with previous gestational diabetes mellitus have a long term increased risk to develop diabetes. Type 1 diabetes developed mainly in the first year postpartum while the risk to develop type 2 diabetes extends over several years. Presence of islet antibodies predicts type 1 diabetes to 100%. Type 2 Diabetes was best predicted by the insulin requirement during pregnancy (White-class) and BMI. Screening hsCRP in postpartal serum samples did not provide additional information for the risk assessment.

## 389

**2 h glucose measurement: is it in fact a gold standard for posprandial hyperglycemia?**
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**Background and aims:** The WHO/ ADA criterion refers to the 2.h values for the evaluation of postprandial hyperglycemia. Many multicenter projects carried out are based on these criteria. While the diagnosis criteria regarding the fasting plasma glucose levels is intensively debated nowadays, we interrogate in this study the adequacy of the 2.h measurement at the oral glucose tolerance test as the gold standard.
**Materials and methods:** 270 subjects (45.1 ± 12.4 years old, female/male: 189/81) without a previous diagnosis of diabetes who had a FPG< 126 mg/dl at the first screening were invited to undergo a 75 gr OGTT for the second step of screening. The glucose oxidase method is used for the measurement of the venous plasma glucose at the 0., 30 min, 60 min, 90 min, 120, 180 min, 240 min and 300 min of OGTT. The levels at the 60., 90. and 120. min were used for the evaluation of postprandial hyperglycemia (>140 mg/dl). The values at the 180., 240. and 300. min are considered for the evaluation of reactive hypoglycemia. Statistical analysis was carried out by using the chi-square test and Student-t test.
**Results:** According to WHO/ADA criteria, 80.2% had normal glucose tolerance, 12.2% had impaired glucose tolerance and 7.6% had diabetes

regarding the 2.h glucose levels during OGTT. When 60. and 90. minutes glucose levels are considered, 37.5% had at least one of these glucose values over 200 mg/dl, furthermore 30% of these patients were considered normal according to the 2.hr glucose. This difference was statistically significant ($p<0.01$). When the cut-off level for postprandial hyperglycemia is considered as 180 mg/dl, 37% of patients at the 60.min, 21.8% at the 90.min and 9.6% at the 120.min had exceeded this upper limit ($p<0.01$ vs $p<0.05$). When this limit is decreased to 140 mg/dl, 52% of patients at the 60.min, 37% at the 90.min and 19% at the 120 min exceeded this limit ($p<0.05$). The percent of patients with plasma glucose values less than 70 mg/dl was highest at 180 min during the OGTT ($p<0.05$).
**Conclusion:** Our results show that the 2.h values are not solely suggestive of postprandial hyperglycemia and that the glucose values at the 60 min and/or 90 min should be determined. As to the evaluation of reactive hypoglycemia, the 180 min values should be considered.

# PS 16
# Prediction of Type 2 diabetes

## 390

**Main predictors of metabolic syndrome and Type 2 diabetes development in the historical cohort of the Brisighella Heart Study: a 16-years follow-up**

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**Background and aims:** It is well known that both metabolic syndrome and type 2 diabetes mellitus are strong risk factors for cardiovascular diseases and that their incidence is drammatically increasing in the Western countries. The aim of this study is to evaluate and quantify the relative role of different biological parameters in the long-term development of metablic syndrome and type 2 diabetes mellitus in an Italian rural population sample.
**Materials and methods:** The Brisighella Heart Study (1972–2003) is a prospective, population-based longitudinal epidemiological cohort involving 2939 randomly selected subjects, aged 14 to 84 years, resident in the Northern Italian rural town of Brisighella. For this study, we selected 1442 age-matched adult subjects (M:712, F:730) consecutively visited during the last four-yearly BHS surveys (1988–2004), with comparable baseline BMI, fasting plasma glucose and smoking habit. Patients affected by type 1 diabetes were previously excluded form the study. The Cox regression analysis was used to determine the independent prognostic significance of age, BMI, FPG, Blood Pressure, total cholesterolemia, triglyceridemia, HDL-Cholesterolemia and uricaemia for metabolic syndrome and type 2 diabetes development on an 8-year long follow-up.
**Results:** In our population sample, FPG and uricaemia appear to be the best predictor of metabolic syndrome, while FPG and HDL-C in men. Age appears to be a significant predictor of type 2 diabetes (but not for metabolic syndrome) when inserted alone in the model ($p=0.007$), but it is completely not relevant when adjusted for baseline BMI and or FPG. Among subjects affected by IFG, the diabetes incidence/year has been estimated to be 5.3% for men and 3.9% for women ($p=0.009$). In the whole population sample, basal glycaemia values under 110 mg/dL have not shown to be significant long-term predictors of diabetes development, while BMI was the best predictor, especially in men.
**Conclusion:** Our findings confirm the relevant role of IFG and BMI as predicting parameters of type 2 diabetes development in the Brisighella population, while FPG appears to be the only significant predictor of metabolic syndrome in both sexes. The evaluation of the best metabolic syndrome and diabetes predictors in each population should help to identify effective approaches for prevention.

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**Forecasting outcomes in people with Type 2 diabetes: an evaluation of two different cardiovascular risk functions**

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**Background and aims:** The Framingham risk function was derived from a study that included a limited number of diabetes patients (4% of 5573), and has been shown to underestimate the risk of vascular disease events in patients with type 2 diabetes. An alternative risk function has been derived from data from the UKPDS, a prospective study in newly diagnosed type 2 diabetes patients. Our study compared event frequency predictions using the Framingham and UKPDS risk functions.
**Materials and methods:** UKPDS and Framingham equations predicting coronary heart disease (CHD) and stroke events were coded into the Eastman NIDDM model. Event predication was compared at baseline and after a 10% reduction in HbA1 c, systolic blood pressure (SBP) or total cholesterol to high-density lipoprotein cholesterol ratio (TC:HDL-C). Simulations were run for a population of 10,000. Baseline variables were as described in the Eastman model, but with age fixed at 52 yr and a simulation study period of 20 yr.
**Results:** The table lists the number of CHD and stroke events predicted by each set of risk equations. The UKPDS functions predicted 72% more CHD events and 143% more strokes than the Framingham functions. Both

models predicted fewer events after a 10% reduction in HbA1 c, SBP or TC:HDL-C. However, the UKPDS consistently predicted more events than Framingham. For example, following a 10% reduction in SBP, the UKPDS model predicted 2.8% fewer events compared with baseline and 88% more events compared with the Framingham model.

**Vascular events predicted by two different risk functions**

| Risk function | Baseline | −10% HbA1c | | −10% SBP | | −10% TC:HDL-C | |
|---|---|---|---|---|---|---|---|
| | Events | Events | Δ(%) | Events | Δ(%) | Events | Δ(%) |
| UK CHD | 5441 | 5024 | −7.7 | 5290 | −2.8 | 5250 | −3.5 |
| F CHD | 3169 | 3065 | −3.3 | 2820 | −11.0 | 3000 | −5.3 |
| Δ CHD | 2272 (72%) | 1959 (64%) | | 2470 (88%) | | 2250 (75%) | |
| UK stroke | 3134 | 3054 | −2.6 | 2850 | −9.1 | 3110 | −0.8 |
| F stroke | 1289 | 1147 | −11.0 | 1000 | −22.4 | 1220 | −5.4 |
| Δ stroke | 1845 (143%) | 1907 (166%) | | 1850 (185%) | | 1890 (155%) | |

UK: UKPDS; F: Framingham; SBP: systolic blood pressure; TC:HDL-C: total cholesterol to high-density lipoprotein cholesterol ratio

**Conclusion:** The validity of a model's output depends on the inherent assumptions, including choice of risk equation. Because the UKPDS risk functions were based on a type 2 diabetes population, they are more appropriate than the Framingham functions. These findings will have a notable effect on forecasting costs and outcomes as type 2 diabetes models driven by Framingham may be less reliable.
*Supported by: AstraZeneca*

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**Abstract withdrawn**

## 393

**Non-invasive urinary myoinositol excretion rate detects glucose intolerance most effectivly for the screening of diabetes mellitus**
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**Background and aims:** The clinical usefulness of urinary excretion rate of myoinositol,a kind of polyol, was studied for the screening of gluccose intolerance including boaderline type caces.
**Materials and methods:** Myoinositol in urine collected before and after 75gOGTT or MTT (meal tolerance test) was determined by the method newly-developed by Asahikasei Co.Ltd. The difference of incremental urinary myoinositol during OGTT [ΔUMI=2 hour−0 hour urinary myoinositol excretion rate (mg/g creatinine)] was compared among groups with various glucose intolerance categorized by Japan Diabetes Society (JDS) and American Diabetes Asociation (ADA,).
**Results:** ΔUMI was significantly correlated with 1 hour plasma glucose (PG), 2 hour PG and AUC in OGTT and MTT, respectiyely, and well differentiated the patterns of glucose intolerance. The cut-off point of ΔUMI during OGTT was determined to be 10 mg/g-Cr by ROC curve analysis. The percentage of positive cases in borderline and diabetic types by 75gOGTT were the best when ΔUMI was adopted, being compared with HbA1 c, 1,5-anhydroglucitol (1,5-AG), glycosilated albumin (GA) or urinary glucose (Table). The similar results were obtained when MTT was performed.
**Conclusion:** ΔUMI well reflected and differentiated the degree of glucose intolerance, indicating the clinical usefulness for the screening of diabetes mellitus, especially in mild cases including IGT and/or IFG.

Various indicies and their positivity (%)

| 75gOGTT | ΔUMI | HbA1 c | 1,5-AG | GA | Urinary-glucose |
|---|---|---|---|---|---|
| Normal Glucose Tolerance (n=429) | 14.0 | 0.6 | 7.1 | 3.3 | 7.2 |
| Borderline-type (n=72) | 63.9 | 18.6 | 29.2 | 18.1 | 45.8 |
| Diabetic-type (n=51) | 100.0 | 61.9 | 72.0 | 64.7 | 96.1 |

## 394

**Associations of lipoprotein measures with the incidence of Type 2 diabetes in the Insulin Resistance Aterosclerosis Study (IRAS)**
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**Background and aims:** Subjects with type 2 diabetes (T2DM) have smaller LDL and HDL particles in addition to higher triglycerides and lower HDL cholesterol. Previous work has suggested that elevated insulin resistance, blood pressure, and triglycerides and lower HDL cholesterol precede the onset of T2DM. In one previous report small dense LDL predicted the incidence of T2DM. However no study has examined heterogeneity within lipoproteins in a population in which insulin resistance was directly measured. This abstract presents associations between lipoprotein measures and the incidence of type 2 diabetes adjusted for both demographics and insulin resistance.
**Materials and methods:** The IRAS is a multi-center, multi-ethnic group study from which frozen plasma samples from baseline subjects were made available for detailed lipoprotein subclass particle analysis by nuclear magnetic resonance (NMR) spectroscopy. Particle concentrations of large, medium, and small VLDL, LDL, and HDL subclasses, along with mean VLDL, LDL, and HDL particle sizes were measured by NMR at LipoScience (Raleigh, NC). Of 830 subjects who were non-diabetic at baseline and returned 5 years later for a follow-up exam, 130 (15.7%) developed diabetes. Insulin sensitivity ($S_I$) was measured by frequently sampled intravenous glucose tolerance test.
**Results:** The following NMR measures, each with the odds ratio for a 1 standard deviation increment and 95% confidence interval, were significantly associated with T2DM incidence in demographically adjusted logistic regression: VLDL particles: 1.23 (1.02, 1.49), Large VLDL/Chylos: 1.46 (1.22, 1.73), LDL particles: 1.49 (1.24, 1.78), Small LDL: 1.48 (1.24, 1.77), Medium Small LDL: 1.50 (1.25, 1.80), Very Small LDL: 1.47 (1.23, 1.76), Large HDL: 0.61(0.48, 0.77), Small HDL: 1.25(1.03, 1.52), VLDL size: 1.48(1.22, 1.78), LDL size: 0.71 (0.59, 0.86), and HDL size: 0.52 (0.41, 0.66). Significant odds ratios for chemically-measured lipid parameters were: apolipoprotein B (apoB): 1.29 (1.07, 1.56), HDL cholesterol: 0.53 (0.41, 0.68), LDL size: 0.74 (0.61, 0.90), and triglycerides (TG): 1.30 (1.10, 1.55). After adjustment for log transformed $S_I$, all of these measures continued to be statistically significant except VLDL particles, NMR LDL size, apoB, HDL, and TG. Using backwards elimination with all demographic variables and the significant NMR measures listed above entered, VLDL size was positively associated and HDL size was negatively associated. Both measures remained significant even after adjustment for log transformed $S_I$.
**Conclusion:** A broad range of abnormalities in lipoprotein composition (i.e. smaller HDL and larger VLDL particles) precedes the onset of T2DM; these differences are not completely explained by the greater insulin resistance, as measured by $S_I$, present in pre-diabetic subjects.

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**K-value and low insulin secretion in a non-obese Caucasian population predicted glucose tolerance after 25 years**
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**Background and aims:** Insulin resistance and insulin deficiency are proposed as risk factors for impaired glucose tolerance (IGT) and type 2 diabetes (T2DM). We assessed the predictive value of initial parameters for outcome of oral glucose tolerance (OGTT) performed 24.3 ± 2.9 years later in an unselected healthy non-obese population.
**Materials and methods:** K-value of intravenous glucose tolerance test (IVGTT) was determined in 267 healthy subjects (age 31.0 ± 12.0 years, BMI 21.8 ± 2.8 kg/m$^2$). First-phase insulin response to glucose infusion test (GIT) was estimated as incremental 5 or 10 min (ΔI5 or ΔI10) value, and as insulinogenic indices (ΔI5/ΔG5 or ΔI10/ΔG10) adjusted for insulin sensitivity determined by HOMA ((ΔI5/ΔG5)/HOMA-IR).
**Results:** At follow-up, 6 subjects had T2DM and 47 IGT; 214 retained normal glucose tolerance. Insulin sensitivity and early (30 min) insulin response decreased with decreasing outcome OGTT.

2-h blood glucose at OGTT correlated positively with initial age and BMI, and negatively with ΔI5/ΔG5, (ΔI5/ΔG5)/HOMA-IR, and K-value. In multiple linear regression analysis (ΔI5/ΔG5)/HOMA-IR, ΔI10, K-value, age, HOMA-estimate of insulin secretion, and fasting plasma glucose significantly associated with 2-h OGTT blood glucose.
Similar results were obtained comparing differences between subjects with normal and decreased (IGT + T2DM) glucose tolerance.
**Conclusion:** In 267 non-obese healthy subjects, initial K-value and first-phase insulin response to glucose adjusted for insulin sensitivity, but not insulin sensitivity per se, were strong predictors of OGTT performed 25 years later. Thus, in contrast to obese or other high-risk populations, in lean subjects decreased β-cell function, but not insulin resistance per se, determines future glucose tolerance.

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**Soluble adhesion molecules, thrombomodulin and risk of developing Type 2 diabetes mellitus. Preliminary results from the MONICA/KORA Augsburg study**

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**Background and aims:** Low grade systemic inflammation and endothelial dysfunction are suggested to be involved in the pathogenesis of type 2 diabetes mellitus (DM). Therefore, the aim of the present study was to investigate prospectively the associations between soluble adhesion molecules (E-selectin, sICAM-1) and thrombomodulin as markers of endothelial dysfunction and incident type 2 DM.
**Materials and methods:** Using a case cohort design, a random sample of all non-diabetic subjects with available blood samples and follow-up data on DM (n=6611) who had participated in at least one of three population based MONICA/KORA Augsburg surveys between 1984 and 1995 was drawn. After exclusion of subjects with missing values, the randomly drawn subcohort contained 459 men and 360 women. During a mean follow-up period of 7.8 years, 221 cases (139 men, 82 women) of incident type 2 DM occurred in the whole study population. To analyse associations between markers of endothelial dysfunction and incident DM, hazard ratios (HRs) were estimated by Cox proportional hazards models using the SAS macro ROBPHREG developed by Barlow and Ichikawa (1998).
**Results:** Median concentrations of E-selectin, sICAM-1 and thrombomodulin in the subcohort were 54.1 ng/ml for men, 44.4 ng/ml for women, 810 ng/ml for men, 754 ng/ml for women, and 4.6 ng/ml for men and 4.0 ng/ml for women, respectively. E-selectin and sICAM-1 were significant predictors of type 2 DM in men after adjustment for age, survey, BMI, physical activity during leisure time, smoking habits, alcohol consumption, actual hypertension, ratio of total cholesterol / HDL-cholesterol and uric acid (3rd tertile vs. 1st tertile: HR for E-selectin 2.0, 95% CI: 1.3–3.2, HR for sICAM-1 1.9, 95% CI: 1.2–3.1). In women, associations were weaker particularly for sICAM-1 and they were no longer significant, possibly due to the smaller number of incident DM cases (3rd tertile vs. 1st tertile: HR for E-selectin 1.8, 95% CI: 0.9–3.3, HR for sICAM-1 1.3, 95% CI: 0.7–2.4). Thrombomodulin was not significantly associated with incident type 2 DM in both sexes.
**Conclusions:** Elevated levels of E-selectin and sICAM predicted the development of type 2 DM, particularly in men. These data support the hypothesis that endothelial dysfunction is associated with newly developed type 2 DM.

## 397

**T-344C polymorphism in the aldosterone synthase (CYP11B2) gene and risk for hyper glycaemia in a large population-based prospective study**

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**Backgrounds and aims:** The aldosterone synthase is an enzymatic mitochondrial complex catalysing the key step in the synthesis of aldosterone. It plays a major role in regulating blood pressure. A variation (T-344C) in the promoter region of the CYP11B2 gene has been associated with plasma glucose levels in a population of Chinese and Japanese origin. Most of them were hypertensive. We tested whether this association could be demonstrated in a large French Caucasian cohort with a 3-year follow-up.
**Material and methods:** Subjects took part in DESIR (Epidemiologic Data on Insulin Resistance Syndrome), a cohort of 2576 men and 2636 women from the general population and clinically and evaluated biologically every three years. Age at inclusion was between 30 and 65. Data were available for the inclusion (T0) and the 3-year visit (T3). 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 fasting glycaemia (FG) and increment in FG between T0 and T3, with or without adjustment for age, body mass index and waist-to-hip ratio, in the whole population and after stratification according to gender and hypertensive status.
**Results:** Allelic frequencies were 55% and 45% for T and C alleles respectively and not different among hypertensives and normotensives. The distribution of genotypes was in Hardy-Weinberg equilibrium. The polymorphism was not associated with the presence of hypertension. No significant association was found between the T-344C polymorphism and FG at baseline or increment in FG in the normotensive participants (2223 men and 2257 women at T0). There were no association with baseline values of FG in hypertensive men or women. However, increment in FG over a 3-year period was significantly different according to T-344C polymorphism (−0.06 ± 0.08, 0.20 ± 0.07 and 0.29 ± 0.12 mM for TT, TC and CC genotypes respectively, p=0.031 before adjustment, p=0.024 after adjustment) in hypertensive women. This was not found in hypertensive men. The results were not affected by concomitant treatments.
**Conclusion:** In a large population-based cohort, we detected in hypertensive women an association between a single nucleotide polymorphism in CYP11B2 gene and 1) the fasting glycaemia during the follow-up and 2) the increment of fasting glycaemia between the baseline and the 3-year values. These data are partially in accordance with previously published results on the same topic in a Chinese and Japanese population. However, we were not able to show a similar association for the male subjects.

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**Cystic-fibrosis-related diabetes mellitus (CFRD): patient characteristics in 1160 CF subjects studied by OGT-test-Results from the German-Austrian CF-Diabetes-Study Group**

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**Background and aims:** As life expectancy increases, the prevalence of secondary diabetes in adolescents and adults with cystic fibrosis is on the rise. Despite numerous indications that diabetes in CF is associated with impaired pulmonary function, more frequent infections and earlier death, both screening for altered glucose metabolism and the preferred therapeutic approach are still controversial.
**Materials and methods:** In Germany and Austria, a multicenter CF-diabetes study group aims at yearly OGT screening tests in all CF patients starting at age 10. By March 2004, a total of 1902 OGT-tests from 1160 CF patients (mean age 19.7 years, 615 males, 545 females) were available for analysis. OGT-tests were performed at the participating centers and analyzed based on WHO criteria. SD-scores for height, weight and BMI were calculated based on recent multicenter German reference data including 34422 healthy children and adolescents.
**Results:** Based on the first OGT-test, 807 subjects displayed normal glucose tolerance, 73 impaired fasting glucose, 174 impaired glucose tolerance and 106 were classified as diabetes. NGT subjects were significantly younger compared to patients with diabetes (18.9 versus 21.7 years, p<0.0001), however 40% of CF subjects with a diabetic OGT-test were younger than 18 years of age. Kaplan-Meier analysis revealed that diabetes occurred earlier in female compared to male subjects (p<0.01, Wilcoxon). Only 46% of CF subjects with a diabetic OGT-test had elevated fasting glucose levels. Diabetic OGT-tests were associated with lower SD-scores for weight (−1.24 versus −0.89, p=0.01), and lower body-mass-index (−0.82 versus −0.62, p=0.01). SD-scores for weight and for BMI were negatively correlated to 2-hour-blood glucose (p<0.003 and p<0.02). A multiple regression analysis revealed that age, gender and glucose metabolism were significant predictors for anthropometric measurements in CF patients. In 568 patients, repeat OGT-tests were performed due to pathologic results in the first test or clinical judgment, the mean interval was 1.1 years. Out of 73 CF subjects with a first diabetic OGT-test, diabetes was confirmed in 34 (47%) by a second OGT-test, while 15 (21%) displayed impaired glucose tolerance, 3 (4%) impaired fasting glucose and 21 (28%) normal glucose tolerance. Only 13 out of 346 patients with a normal glucose test (3.7%) displayed a diabetic

repeat test, 19 (5.5%) progressed to IFG and 37 (10.7%) to IGT during the observation period.
**Conclusion:** This large multicenter study from Germany and Austria confirms the high prevalence of abnormal glucose tolerance in the second and third decade of life in CF patients. Subjects with pathologic glucose metabolism are underweight compared to CF patients with normal glucose tolerance. In individual subjects the OGT-test displays a high variability, diabetes has to be confirmed by two independent OGT-tests prior to the initiation of therapy. These data represent the screening phase of a prospective intervention study comparing oral agents (repaglinide) to insulin therapy in cystic fibrosis.
*We like to thank especially the German Cystic Fibrosis Foundation and also NovoNordisk and the CF-patients group of Saarbrücken for funding this multicentre study. We also like to thank all the participating centres of the CF-Diabetes-Study Group.*

# PS 17

# Prevention of Type 2 diabetes and complications

## 399

**National diabetes prevention – evaluation of the TUMAINI diabetes prevention programme (TPP)**
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**Background and aims:** Diabetes is one of the main threats to human health in this century. To prevent the personal and socio-economic burden of diabetes effort to prevent the disease needs to start before the onset of diabetes and address all susceptibility factors. Recent studies have shown that prevention of diabetes is possible. The translation of the study strategies into practical national prevention programmes accessible by the average population are needed. The here evaluated programme concept was chosen from the Germany Ministry of Health as strategy for a National Diabetes Prevention Program.
**Materials and methods:** Combining the essentials of the DPP and DPS with strategies of behaviour modification and group intervention programmes we developed the TUMAINI-Diabetes Prevention Programme (TPP). Than we introduced the program to 1176 healthy people with family history of diabetes for questionnaire based (1= excellent/helpful, 5=disagree/not helpful) evaluation for: A: overall evaluation, B: convenience, C: practicability, D: motivation to change, E: good care for the programme steps and the Health miles positive feedback system.
**Results:** The TPP is a group session based program and consists of 3 programme steps:: 1: Contact and screening for the identification of the subjects; 2. Training program for diabetes prevention as general intervention and motivation step and 3. Continuous follow up to maintain motivation, evaluation and quality control using different delivery channels. 528 People answered the survey. The risk screening was highly scored as well as the people wanted a continuous risk monitoring and follow up (A: 1,70, B: 1.92, C: 2,10, D: 2,10, E: 1,86). The Health miles was seen as perfect way for keep continuous motivation and got the best scores (p=0,008). Written information and telephone counselling was seen as less successful. People above 60 scored internet intervention much better than below (p=0,003). Overall evaluation of the programme was for people with BMI >27 sign. better than below (p=0,013).
**Conclusion:** The TPP could be realized decentralized, is based on small groups, and easy to evaluate. Such a programme is widely accessible, using a success orientated intervention and personalized intervention strategies and can be implemented as National Prevention Program. A positive feedback system like the TUMAINI Health miles system is a continuous supportive motivator.
*Supported by: TUMAINI-Institut*

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**Health economic evaluation of the STOP-NIDDM trial from a German perspective**
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**Purpose:** The Study To Prevent Non-Insulin-Dependent Diabetes Mellitus (STOP-NIDDM) showed that treatment with acarbose in patients with prediabetes (impaired glucose tolerance, IGT) over a mean 3.3 years significantly reduced the risk of diabetes (HR 0.75 [95% CI 0.63–0.90]; p=0.0015) and cardiovascular events (HR 0.51 [95% CI 0.28–0.95]; p=0.03) compared to placebo. Based on the STOP-NIDDM trial, a health economic model was developed to evaluate the impact of acarbose treatment in IGT patients for Germany.
**Methods:** A cost-effectiveness model from the perspective of the German Statutory Health Insurance (GKV) was developed. Only direct healthcare resource costs were considered, including those costs associated with management of diabetes and cardiovascular events (CVEs). For acarbose

treated patients, the costs of GP vists for IGT management were considered, while these costs were not included for the placebo group. Analyses were conducted for the total STOP-NIDDM patient population and three subgroups at high risk for diabetes, at high risk for CVEs and at high risk for both. The subgroups were defined by applying specific risk scores.
**Results:** For the total STOP-NIDDM population a cost-effectiveness ratio of Euro 772 per case of diabetes prevented over 3.3 years was estimated. In the base case analyses for the subgroups, cost savings per treated patient of Euro 42 (high diabetes risk), Euro 674 (high CHD risk), and Euro 408 (high diabetes and CHD risk) over 3.3 years were calculated. The results were generally robust to sensitivity analysis.
**Conclusion:** In addition to the significant clinical benefits observed with acarbose therapy in pre-diabetic patients, the cost-effectiveness model estimates potential cost savings to the German healthcare system in those patients at high risk for diabetes and / or cardiovascular events. For the total population, acarbose therapy was estimated to be cost-effective.

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**Laparoscopic gastric banding prevents Type 2 diabetes and arterial hypertension in morbid (grade 3) obesity: a 4 years study**
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**Background and aims:** Obese subjects are at risk of type 2 diabetes (T2DM) and arterial hypertension, and lifestyle changes and pharmacologic treatment with metformin have been shown to prevent type 2 diabetes in obese IGT subjects.
**Materials and methods:** In this study we evaluated glucose tolerance (GT, through OGTT, 75 g), fasting insulin and blood glucose, HOMA index and arterial hypertension at baseline and after 4 years in subjects treated with laparoscopic gastric banding (LAGB) and in subjects refusing LAGB (Table 1).
**Results:** Subjects undergoing LAGB had a significant decrease of BMI, HOMA index, and a significant improvement in GT (4 T2DM improved to IGT or NGT, 10 IGT improved to NGT, and only 1 subject progressed from NGT to IGT; no new cases of T2DM occurred, and only 1 subject developed hypertension. Subjects refusing LAGB had no change of BMI, HOMA index, and 7 subjects had deterioration of GT (4 new cases of T2DM, 3 progression from NGT to IGT; 3 IGT improved to NGT, and 1 T2DM improved to IGT) and of hypertension (7 new cases). (* improved vs basal conditions (student's t test or chi-square test); § deteriorated vs baseline (chi-square test). Changes of GT and of hypertension were significant between the 2 groups of subjects ($p < 0.05$ and $<0.01$, respectively). At stepwise regression analysis, change of HOMA predicted change of GT and of hypertension.
**Conclusion:** These data indicate that laparoscopic gastric banding prevents type 2 diabetes and arterial hypertension in morbid obesity at least for 4 years.

| | LAGB | | no-LAGB | |
|---|---|---|---|---|
| | baseline | follow-up | baseline | follow-up |
| subjects | 52 | | 31 | |
| age (years) | 47.3 ± 1.58 | | 47.9 ± 1.79 | |
| BMI (kg/m$^2$) | 46.6 ± 1.35 | 37.1 ± 0.92 | 46.8 ± 1.77 | 46.8 ± 2.41 |
| hypertension | 23 | 15 | 21 | 28 § |
| glucose tolerance (NGT/IGT/T2DM) | 26/17/9 | 37/10/5 * | 12/9/10 | 12/6/13 |
| insulin (μU/ml) | 18.1 ± 1.73 | 11.4 ± 1.35 * | 15.3 ± 1.63 | 17.9 ± 2.22 |
| glucose (mmol/l) | 6.2 ± 0.32 | 5.4 ± 0.14 * | 6.5 ± 0.74 | 6.7 ± 0.87 |
| HOMA (gluc.ins/22.5) | 5.2 ± 0.06 | 3.2 ± 0.51 * | 4.3 ± 0.06 | 4.4 ± 0.58 |

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**PPAR-alpha activator can prevent diabetes with increased plasma level of adiponectin**
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**Background and aims:** Fenofibrate is one of PPAR alpha activators. One of fenofibrate main actions has been suggested to increase beta-oxidation of fatty acids in the mitochondria of hepatocyte. We have found that fenofibrate can prevent diabetes in obese diabetic rat model by reducing body adiposity. The hepatic mechanism for reducing adiposity is supposed that fenofibrate increase energy expenditure by increase of uncoupling via de novo expression of UCP-3 in hepatocyte. We hypothesized that decreased adiposity by PPAR alpha activator may result in increasing plasma level of adiponectin, which also can contribute the anti-diabetic effect. We performed this study to corroborate our hypothesis by measuring body weight and plasma adiponectin level in obese diabetic rats.
**Materials and method:** Ten week aged OLETF rats (n=30) were randomly divided into three groups. One group (n=10) was feed freely (free feeding group). Second group (n=10) was feed with fenofibrate (320 mg/kg) (fenofibrate group). Third group (n=10) was feed with the same amount of food intake as fenofibrate group has taken (paired feeding group). The body weight was measured in every week. At the age of 39 weeks, blood samples were drawn via tail veins and plasma levels of glucose and adiponectin were analyzed.
**Results:** The body weight of fenofirate group (482 ± 23 g) was significantly lowered compared with free feeding group (691 ± 70 g) or paired feeding group (686 ± 41g) ($p < 0.05$). The plasma glucose level of fenofibrate group (7.96 ± 1.02 mmol/L) was also lowered than free feeding group (26.48 ± 7.11 mmol/L) and paired feeding group (15.35 ± 5.60 mmol/L) ($p < 0.05$). The plasma level of adiponectin in fenofibrate group (4.54 ± 1.22ug/ml) was significantly increased compared with that of free feeding group (2.51 ± 0.49 ug/ml) or paired feeding group (2.46 ± 0.57 ug/ml) ($p < 0.05$).
**Conclusions:** Fenofibrate treatment can prevent the development of diabetes in OLETF rats with decreasing weight gain. The increased production of adiponectin is associated with anti-adiposity effect of fenofibrate.

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**Dexametazone induced glucose intolerance predicts the development of diabetes in relatives of Type 2 diabetic patients 10 years later**
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**Background and aims:** Ten years ago we investigated 20 normoglycemic relatives of Type 2 diabetic patients and 20 subjects without a family history of diabetes. The subjects were tested before and following 5 days treatment with Dexametazone (DEX)(4 mg per day). During the steroid treatment 7 relatives developed severe glucose intolerance (defined as 2 h OGTT PG > 10.0 mmol/l), and of these 4 had a diabetic glucose tolerance. All subjects developed severe insulin resistance during steroid treatment but a reduced beta cell response was the main reason for the development of steroid induced glucose intolerance. All subjects had normal glucose tolerance following withdrawal of the steroid treatment.
The aim of the present study was to re-test the same 40 subjects now 10 years latter with respect to present glucose tolerance and to identify factors responsibly for a possibly deterioration in glucose tolerance seen now.
**Materials and methods:** Oral (OGTT) and intravenous (IVGTT) glucose tolerance tests the latter for measurements of the acute insulin response to glucose (AIR) and insulin sensitivity (Minimal Model). Twenty relatives and 18 control subjects accepted to be re-examined.
**Results:** Of the 7 previous "severe DEX glucose intolerant" relatives 4 have now developed frank diabetes (two with previous unknown diabetes) (Fischer's exact test $p<0.04$). Three of the 4 relatives had a diabetic glucose tolerance during DEX treatment. One control subject has developed diabetes as judged from the fasting the PG but 2 h OGTT PG was normal. Body weight has increased substantial in both groups during the 10 years (relatives: 92.0 vs 76.1 kg, controls: 84.6 vs 71.6 kg). The 7 previous DEX glucose intolerant relatives now have an absolute decreased AIR as compared both to the 13 "DEX glucose tolerant relatives" and the control subjects (38 vs 168 vs 210 pmol/l, $p< 0.05$) whereas changes in insulin sensitivity seems to have no influence on the development of diabetes.
**Conclusion:** Development of glucose intolerance during steroid treatment predicts the development of diabetes 10 years later in relatives of Type 2 diabetic patients. The development of diabetes may be due to deterioration in beta cell function over time.

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**Prevalence of insulin resistance in morbid obesity in young Europeans**
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**Background and aims:** Morbid obesity is associated with insulin resistance which precedes the development of type 2 diabetes mellitus (T2DM) in adults. However, data on the prevalence of impaired glucose tolerance (IGT), T2DM and its correlation with insulin resistance in children and adolescents are sparse. We therefore determined the prevalence of IGT and measures of insulin action and -secretion in 73 obese children and adolescents.
**Materials and methods:** Participants underwent a two-hour oral glucose-tolerance test (OGTT: 1.75 mg of glucose per kilogram of body weight). Insulin sensitivity was estimated by the oral glucose insulin sensitivity index (OGIS), a parameter obtained from OGTT that describes insulin action in dynamic conditions (i.e. under glucose stimulation of the beta cell). OGIS has been extensively validated against the glucose clamp. Insulin sensitivity was evaluated with homeostatic model assessment (HOMA-IR), insulin resistance being defined by HOMA-IR > 3.2 (mmol/l · μU/ml) and OGIS values > 436 ml $min^{-1}$ $m^{-2}$. Basal beta-cell function was determined with HOMA-B, while the insulinogenic index (suprabasal insulin divided by suprabasal glucose, both at 30 min) was used as an index of dynamic beta cell function. β-cell sensitivity-secretion relationship was described by the disposition index.
**Results:** Only morbidly obese children and adolescents (n=73, age range: 6–18 years, BMI 32 ± 6.4 $kg/m^2$) were included. IGT was found in 4 (5.5%) patients, whereas no overt T2DM was detected. HOMA-IR (5.5 ± 3.4) and OGIS (400 ± 65.3) revealed decreased insulin sensitivity. HOMA-IR correlated significantly with BMI (R=0.31, p<0.03). HOMA-B was 704.3 ± 505.4, Insulinogenic index 1.23 ± 0.8 (μU/ml)/(mg/dl), reflecting enhanced insulin secretion. Disposition index was 1126 ± 1206, indicating compensation of insulin resistance through enhanced insulin secretion. HOMA-IR and OGIS were tightly correlated in this population (R=0.55, p<0.001).
**Conclusion:** Obesity is correlated with insulin resistance also in obese youth. Insulin resistance and IGT are frequent among obese children and adolescents. Obese young are still able to compensate for insulin resistance by increasing insulin secretion. These data underline the need of early treatment in order to prevent β-cell failure and development of overt DMT2.

# PS 18
# Exocytosis in beta cells

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**The time-dependent potentiating signal responsible for the second phase of glucose-stimulated insulin release increases the size of the docked granule pool**
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**Background and aims:** In glucose-stimulated biphasic insulin secretion the 1st phase is thought to be due to exocytosis of an "immediately releasable" pool of docked granules and the 2nd phase to a time-dependent potentiating signal. The mechanisms responsible for the 2nd phase are unknown. The aim of this study was to determine whether the increased rate of insulin secretion during the 2nd phase was associated with an increase in the number of granules docked at the β-cell plasma membrane.
**Materials and methods:** Electron micrographs were prepared from isolated pancreatic islets subjected to rapid fixation under control (2.8 mM glucose), stimulated (16.7 mM glucose) and other conditions. Classical quantitative morphometric techniques were applied and at least 10 β-cells examined for each condition. Insulin secretion was measured under perifusion conditions by RIA.
**Results:** The glucose-activated time-dependent potentiating signal results in an increase in the number of docked granules. We found that the "average" rat β-cell contains 11,136 granules. Under control conditions with 2.8 mM glucose, 6.4% of the β-cell granules (712) were docked. After 40 minutes in the presence of 16.7 mM glucose 10.2% of the granules (1135) were docked. This significant increase occurred at a time when the rate of exocytosis was elevated and demonstrates that the rate of granule docking exceeds the rate of exocytosis. Therefore, the number of docked granules is not rate limiting for 2nd phase release. Under the same conditions but with nitrendipine present to block exocytosis, there was no further increase in the size of the docked pool, suggesting that there is a limit to the number of docking sites. These data were confirmed by determining the number of docked granules per unit length of membrane. Under control conditions there were 4.3 ± 0.6 docked granules/10 μm of membrane and after stimulation by glucose there were 6.5 ± 0.8 docked granules/10 μm membrane, P<0.05.
Treatment of islets for four hours with 0.68% fatty acid-free BSA results in a massive enhancement of glucose-stimulated insulin release (up to 10-fold). Under these conditions stimulation with 16.7 mM glucose for 40 minutes decreased the size of the docked pool (1.9 ± 0.2 docked granules/10 μm of membrane compared with 6.1 ± 0.7 docked granules/10 μm of membrane for the controls, P<0.01). Therefore, the rate of docking failed to keep up with the extraordinarily high rate of exocytosis. However, under the same conditions but in the presence of nitrendipine the docked granule pool was increased to the same level as control islets also exposed to 16.7 mM glucose in the presence of nitrendipine (6.5 ± 0.5 docked granules/10 μm of membrane compared with 6.6 ± 0.6 granules/10 μm of membrane). This similarity of the size of the docked granule pool under different conditions again suggests that the number of available docking sites is limited.
**Conclusion:** The 2nd phase of glucose-stimulated insulin release is associated with an increase in the number of granules docked at the plasma membrane. The increased availability of docked granules for release may be the cause of the increased rate of 2nd phase secretion. Also, as under normal glucose-stimulated conditions the number of docked granules is not rate limiting for release, the rate-limiting step that controls the 2nd phase of insulin release must be the rate at which the docked granules are prepared for release.
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## 406

**Heterogeneous release of foreign cargo proteins from beta cells**
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**Background and aims:** Foreign cargo proteins have previously been used to study protein trafficking and secretion in pancreatic beta cells. When different groups used fluorescent cargo proteins to study secretion, results

differed considerably even when similar cell types and techniques were used. While some groups reported that fluorescent cargo proteins were completely released following membrane fusion, others reported frequent retention. Why these differences occurred remains unexplained.
**Materials and methods:** Primary cultured rat pancreatic beta cells were prepared by standard methods. Adenovirus was used to introduce genes encoding fluorescent cargo proteins. 36–48 hours following transduction, cells were monitored with total internal reflection fluorescence (TIRF) microscopy. RIN cells were also used for some experiments (see below). Transient application of elevated extracellular potassium stimulated vesicle fusion. Experiments were conducted at 37 C with constant exchange of the bath solution. Quickchange (Stratagene) was used for mutagenesis.
**Results:** In parallel experiments, primary cultured pancreatic beta cells were labeled with one of four fluorescent cargo proteins: insulin C-peptide fused to either (1) emerald GFP (C-emGFP) or (2) TIMER (C-TIMER), (3) rat islet amyloid polypeptide fused to EGFP (rIAPP-EGFP) or (4) syncollin fused to EGFP (syncollin-EGFP). For all probes, fluorescence appeared punctate with similar characteristics. Thus, resting cells labeled with different fluorescent cargo proteins were indistinguishable in monochromatic TIRF micrographs. For all probes, potassium stimulated sudden fluorescence changes for a fraction of membrane-proximal vesicles. Surprisingly, these fluorescence changes varied widely, ranging from rapid disappearance to persistent brightening. Unexpectedly, each probe displayed a characteristic response demonstrating that different fluorescent cargo proteins behave differently in pancreatic beta cells. To explain these results we proposed and tested three hypotheses: (1) Different fluorescent cargo proteins partition differently between the dense core and halo within insulin vesicles, *and* the dense core dissolves slowly or incompletely following membrane fusion. (2) Cysteine residues introduced by cloning influence retention of a fluorescent cargo protein following membrane fusion. (3) The entire sequence of amino acids introduced by cloning influences retention of a fluorescent cargo protein following membrane fusion. To test these hypotheses, we used rIAPP-EGFP, a probe that initially displayed only persistent brightening following membrane fusion. We compared this behavior to vesicles that were labeled with (1) the same rIAPP-EGFP but expressed in primary cultured beta cells from mice lacking CPE, an enzyme essential for formation of the insulin dense core, (2) a variant of rIAPP-EGFP with a C-to-S substitution (RIN), and (3) a variant of rIAPP-EGFP lacking amino acids introduced by cloning (RIN). Labeled vesicles brightened and persisted in all of these experiments *except* when the amino acids introduced by cloning were completely removed. Then, the labeled vesicle brightened transiently and often disappeared.
**Conclusion:** From these results, we conclude that amino acids introduced during cloning can alter release of foreign proteins including fluorescent cargo proteins. These results should be interesting for groups using similar probes to study trafficking and/or exocytosis from pancreatic beta cells.
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**Role of phosphoinositides in the regulation of insulin exocytosis**
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**Background and aims:** Phosphoinositides (PI) are important signaling molecules involved in the regulation of vesicular trafficking in different cell systems. In this study we investigated the role of PI and PI-binding proteins in the control of insulin exocytosis.
**Materials and methods:** We used intact or streptolysin-O permeabilized insulin-secreting INS-1E cells transfected with plasmids that allow either the overexpression of the selected proteins or the reduction of their endogenous levels by RNA interference.
**Results:** We found that the introduction of 10 μM phosphatidylinositol 4-phosphate (PI4P) or phosphatidylinositol 4,5-biphosphate (PI4,5$P_2$) in permeabilized INS-1E cells increases by about two folds the secretory response triggered by 10 μM $Ca^{2+}$ (205±41% (n=6) and 189±28% (n=4) of control, respectively). In contrast, phosphatidylinositol 3,4,5-triphosphate at the same concentration had no significant effect on exocytosis. To clarify the mechanism of action of PI, we investigated the involvement of two potential PI targets, the $Ca^{2+}$-dependent activator protein for secretion 1 (CAPS1) and phospholipase D1 (PLD1) in the regulation of insulin exocytosis. Overexpression of CAPS1 did not significantly affect secretion of intact INS-1E cells stimulated by glucose and cyclic AMP-raising agents. However, in INS-1E cells expressing a small interfering RNA that specifically reduces endogenous levels of CAPS1, hormone release elicited by secretagogues was diminished by 49±11% (n=4). Silencing of CAPS1 decreased also the secretory response of streptolysin-O permeabilized INS-1E cells. $Ca^{2+}$-induced secretion was diminished by 62±11% (n=4) and hormone release in the presence of PI4P was reduced by 58±10% (n=3). Overexpression of wild type PLD1 enhanced secretion elicited by glucose and cyclic AMP raising agents (169±22% of control). In contrast, transfection of the cells with a PLD1 dominant negative mutant inhibited stimulus-induced exocytosis by 27±4% (n=3).
**Conclusions:** Our data indicate that PI are involved in the regulation of β-cell secretion and suggest that at least part of their effects on insulin exocytosis could be exerted through the activation of CAPS1 and PLD1.
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**Analysis of the role of Munc18a in insulin secretion**
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**Background and aims:** The Munc18 proteins play a complex and enigmatic role in the regulation of exocytosis. This familiy of proteins has been well established to bind with high affinity to syntaxin and to be mutually exclusive of SNARE complex formation. Structural analysis has demostrated that Munc18a maintains syntaxin 1 in a closed conformational state preventing its interaction with SNAP25 and VAMP2. Furthermore, overexpression of Munc18 proteins reduces neurotransmitter release and blocks vesicle trafficking events. However, loss of Munc18a in knockout mice results in the complete inhibition of vesicle trafficking. Thus, both positive and negative roles for Munc18 have been suggested. Similarly, the same dual effect appears to occur for insulin secretion. We have used the technique of RNA interference to knock-down the expression of Munc18a in the pancreatic β cell line INS1E in order to address the specific role of Munc18a in insulin secretion.
**Materials and methods:** All experiments were performed in the rat pancreatic β cell line INS1E. For the knock-down of Munc18a, cells were transfected with the RNAi expression vector pSUPER-GFP-Munc18a, which encodes an shRNA sequence specific for rat Munc18a, or with the pSUPER-GFP empty vector as a control, using Lipofectamine 2000 as the transfection reagent. For the secretion studies, cells were co-transfected with pSUPER-GFP-Munc18a (or pSUPER-GFP as a control) and a human growth hormone expression vector. Levels of secreted human growth hormone were measured with a human growth hormone ELISA kit in co-transfected INS1E cells stimulated with 16.7 mM glucose + 0.1 μM PMA + 5 μM forskoline + 0.1 mM IBMX 3 days after co-transfection.
**Results:** The plasmid was succesfully employed in this study to decrease Munc18a protein levels in INS1E cells by aproximately 80% as compared to the control. This decrease was shown both by Western blot of transfected GFP-positive cells sorted by FACS and immunofluorescence analysis. Depletion of Munc18a by RNA interference caused a 56% reduction in the secretory response of INS1E cells in Munc 18a-depleted vs. control cells (Munc18a-depleted cells: 5.9 fold stimulation± 0.6; Control cells: 14.3 fold stimulation ± 2.7; mean ± SEM, n=5, $P<0.02$ from 2 independent experiments).
**Conclusion:** Depletion of Munc18a in the INS1E cell line results in a marked decrease in the secretory response of this cell line, suggesting that the presence of this protein is required for the exocytotic process in pancreatic β cells.
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**Evidence that exocytosis per se regulates turn over of the exocytotic protein SNAP-25 by the proteasomal pathway**
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**Background and aims:** SNARE proteins, such as SNAP-25 play a crucial role in exocytosis of insulin. We have tested for regulation by exocytosis per se, using diazoxide and somatostatin as probes, since both compounds reversibly inhibit glucose-induced insulin secretion.
**Material and methods:** Rat pancreatic islets were cultured for 24 h in 5.5 and 27 mM glucose (G). After culture exocytotic proteins were quantified by Western blot and mRNA levels measured by RT-PCR.
**Results:** 24 h exposure to 27 mM G increased the expression of SNAP-25 protein levels (table). When diazoxide (table) or somatostatin (not shown) was present during culture SNAP-25 protein levels were decreased both after 5.5 and 27 mM G. This decrease was not paralleled by any reduction of

SNAP-25 mRNA. In fact, there was an increase after previous diazoxide both after 5.5 (174±22%) and 27 (121±7%) mM G culture. The same results were obtained using isoforms α and β. To investigate whether diazoxide influenced the turnover of SNAP-25 protein we tested effects of the proteasome inhibitors MG 132 and ALLN. Inclusion of these compounds during culture together with diazoxide abolished the reductions in SNAP-25 protein levels (table).
**Conclusion:** Our results indicate that exocytosis per se regulates SNAP-25 and that this is achieved by affecting turn over of the protein.

SNAP-25 protein (% of 5.5 mM G)

| culture (mM G) | 5.5 | 27 | 5.5 + MG132 | 27 + MG132 |
|---|---|---|---|---|
| no previous diazoxide | 100 | 130±8[a] | 113±7 | 136±8 |
| previous diazoxide (325 μM) | 74±4[b] | 76±6[b] | 119±5[c] | 130±8[c] |

[a] $p<0.05$ vs 5.5 mM G; [b] $p<0.05$ vs no previous diazoxide; [c] $p<0.05$ vs no previous MG 132, n=6

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**Impact of sterol response element binding protein-1c on insulin-containing secretory vesicle dynamics**
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**Background and aims:** Accumulation of intracellular triglyceride by pancreatic islet β-cells may contribute to the loss of normal glucose-regulated insulin secretion ('lipotoxicity'). Up-regulation of the lipogenic transcription factor sterol regulatory element binding protein-1 c (SREBP-1 c) has been implicated in mediating these effects. Here we examine the effects of SREBP-1 c overexpression on insulin-containing vesicle movement and plasma membrane fusion.
**Materials and methods:** Vesicle dynamics were imaged in single pancreatic MIN6 β-cells expressing vesicle lumine targeted pH-insensitive yellow fluorescent protein, neuropeptide Y.Venus, by total internal reflection fluorescence microscopy.
**Results:** Over-expression of a constituitively-active form of SREBP-1 c (amino acids -403; SREBP CA), inhibited glucose-stimulated (3 *versus* 30 mM) vesicle movements and reduced the proportion of long excursion events. Moreover, measurements of vesicle density suggest that SREBP CA expression inhibited the recruitment of vesicles to the plasma membrane from the cell interior; a pre-requisite for sustained insulin secretion. Previous results and microarray analysis have shown up-regulation of the mitochondrial uncoupling protein UCP-2 in response to SREBP CA, in parallel with a reduction in cellular ATP content with no evident changes in the expression of the implicated motor proteins (e. g. Kinesin I).
**Conclusion:** Since SREBP-1 c overexpression is associated with decreased glucose-induced increases in ATP, we speculate that this reduction in cellular ATP is likely to be responsible for the inhibitory effect on exocytotic machinery, which may also contribute to the observed inhibition of vesicle movement and insulin secretion in some forms of type 2 diabetes mellitus.

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**Glutamate promotes insulin exocytosis through inhibition of protein phosphatases in pancreatic β-cells (INS-1E)**
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**Background and aims:** In human type 2 diabetes mellitus, loss of glucose-sensitive insulin secretion is an early pathogenetic event. Glucose is the cardinal physiological stimulator of insulin secretion from the pancreatic β-cell, but the mechanisms involved in glucose sensing are not fully understood. A messenger role has been postulated for L-glutamate in linking glucose stimulation to sustained insulin exocytosis in the β-cell, but the precise nature by which L-glutamate controls insulin secretion remains elusive. In the present study, the effects of L-glutamate on the activities of ser/thr protein phosphatases (PPase) and $Ca^{2+}$-regulated insulin exocytosis in INS-1E cells were investigated.
**Materials and methods:** Static insulin release and cellular L-glutamate content measurements were done in INS-1E cells with RPMI-1640 medium containing either 1 mM glucose or 20 mM glucose, without glutamine. Determination of phosphatase activity was done with two different substrates (phosphohistone and phosphorylase *a*) to exclude any substrate-directed artefacts. The statistical probability of differences between groups was analyzed by Student's *t* test or one-way ANOVA, with *post hoc* analysis using Bonferroni's test. For differences within groups, one-way ANOVA was used. $P < 0.05$ was deemed significant.
**Results:** We show that glucose increases L-glutamate contents and promotes insulin secretion from INS-1E cells. L-glutamate, at physiological concentrations, also inhibits enzyme activities of ser/thr PPases in a dose-dependent fashion analogous to the specific PPase inhibitor, okadaic acid. Additionally, L-glutamate and okadaic acid directly and non-additively promote insulin exocytosis from permeabilized INS-1E cells in a $Ca^{2+}$-independent manner.
**Conclusion:** An increase in phosphorylation state, through inhibition of protein dephosphorylation by glucose-derived L-glutamate, may be a novel regulatory mechanism linking glucose sensing to sustained insulin exocytosis.

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**Regulation of PTB nucleocytoplasmic translocation and expression of secretory granule proteins in INS-1 cells**
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**Background:** Pancreatic beta-cells store insulin into secretory granules that undergo exocytosis upon glucose stimulation. Sustained stimulation depletes beta-cells of their granule pool, which must be quickly replaced. We have recently shown that glucose promotes the nucleocytoplasmic translocation of polypyrimidine tract-binding protein (PTB) in beta-cells. Activated cytosolic PTB binds mRNAs encoding secretory granule proteins, thus favoring their stabilization and translation, whereas knockdown of PTB by RNA interference results in the depletion of secretory granules. Previous studies in neuroendocrine PC12 cells demonstrated that PTB phosphorylation by protein kinase A induce its nucleocytoplasmic transport.
**Material and methods:** To elucidate whether a similar mechanism is responsible for the glucose-stimulated nucleocytoplasmic translocation of PTB rat insulinoma INS-1 cells have been treated with a variety of pharmacological agents that modulate the activity of PKA, ERK1/2, and p38 MAPK.
**Results:** Our data indicate that treatments which stimulate PKA activity enhance the phosphorylation and nucleocytoplasmic translocation of PTB also in INS-1 cells, as well as the rapid induction of insulin secretory granule markers ICA512/IA-2 and Chromogranin A.
**Conclusions:** These results suggest that phosphorylation of PTB by PKA may represent a common mechanism by which, in response to various stimuli, different neuroendocrine cells, including beta-cells, promote the rapid post-transcriptional up-regulation of secretory granule proteins, and thereby secretory granule assembly and exocytosis.
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# PS 19
# Ion channel function in islet cells

## 413

**Interaction of membrane potential and intracellular free calcium concentration in beta- cells from of SUR1$^{-/-}$ mice**

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**Background and Aims:** We have shown previously that membrane potential (Vm) and cytosolic free $Ca^{2+}$ concentration ($[Ca^{2+}]_c$) are oscillating in pancreatic beta-cells from mice lacking functional $K_{ATP}$ channels (SUR1$^{-/-}$), but the strong dependence of Vm and $[Ca^{2+}]_c$ on glucose concentration is disrupted. We examined whether $[Ca^{2+}]_c$ and Vm are still coupled in these cells and whether a feedback of $[Ca^{2+}]_c$ on Vm exists.
**Materials and Methods:** Vm was measured with intracellular microelectrodes; ion currents with the patch-clamp technique. $[Ca^{2+}]_c$ was assessed using fura-2. Insulin secretion was determined by radioimmunoassay.
**Results:** In 15 mM glucose Vm (n=53) and $[Ca^{2+}]_c$ (n=11) were oscillating in SUR1$^{-/-}$ beta-cells with similar period and frequency. Administration of 30 mM $K^+$ (n=5) or 10 mM L-arginine (n=4) led to sustained, reversible depolarizations and concomitantly to biphasic increases in $[Ca^{2+}]_c$ (30 mM $K^+$: n=10; peak=705 ± 46 nM; plateau=339 ± 20 nM; 10 mM L-arginine: n=11; peak=614 ± 41 nM; plateau=293 ± 32 nM). Insulin secretion is enhanced in 30 mM $K^+$ (1.02 ± 0.10 ng/(islet*h), n=6; p<0.0005) and 10 mM L-arginine (1.10 ± 0.14 ng/(islet*h); n=5; p<0.005) compared to 15 mM glucose alone (0.48 ± 0.06 ng/(islet*h); n=6). We investigated whether $[Ca^{2+}]_c$ was able to influence Vm. Raising the external $[Ca^{2+}]$ ($[Ca^{2+}]_e$) from 2.5 to 10 mM led to a significant decrease in the fraction of time spent in the plateau phase (FOPP) ($[Ca^{2+}]_e$ 2.5 mM: 33 ± 3%; $[Ca^{2+}]_e$ 10 mM: 24 ± 4%; n=10; p<0.05). This suggests activation of a hyperpolarising current. Accordingly, in perforated-patch experiments an outward current of 7.5 ± 1.2 pA (n=7; p<0.001), inhibitable by D600, could be induced by a train of voltage pulses simulating a burst of action potentials. By contrast, increasing $[Ca^{2+}]_c$ by depletion of intracellular calcium stores by 10 µM cyclopiazonic acid (CPA) led to an increase in FOPP (G15: 44 ± 7% *vs* CPA: 58 ± 5%; n=5; p<0.05). Correspondingly, CPA induced a $Ca^{2+}$ influx which was not inhibited by D600, but was sensitive to removal of extracellular $Ca^{2+}$ (in D600 $[Ca^{2+}]_c$ = 60 ± 5 nM *vs* 26 ± 3 nM in $Ca^{2+}$-free solution with CPA, n=8; p<0.0001 and 53 ± 6 nM without CPA, n=7; n.s.).
**Conclusions:** The sequence of events leading to insulin secretion in SUR1$^{-/-}$ beta-cells (i.e. changes in Vm govern $[Ca^{2+}]_c$ and exocytosis) is in principle the same as in wildtype mice, although Vm is largely uncoupled from glucose metabolism. The results show that $[Ca^{2+}]_c$ influences Vm in two opposite directions, either hyperpolarising by activation of a $Ca^{2+}$-dependent $K^+$ current when $[Ca^{2+}]_c$ is increased directly under the membrane or depolarising by a store operated $Ca^{2+}$ channel when $[Ca^{2+}]_c$ is augmented by release from intracellular stores.
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## 414

**Dexamethasone induced inhibition of insulin secretion depends on expression of SGK1 which alters Kv channel activity**

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**Background and aims:** Glucocorticoid treatment impairs insulin secretion which finally leads to diabetes mellitus. Previously we found that dexamethasone (dex) augments the expression of Kv channels in insulin secreting cells. Repolarizing currents inhibit insulin secretion by counteracting $Ca^{2+}$ influx the major signal necessary for stimulation of release. SGK1 (serum and glucocorticoid regulated kinase 1) has been found to regulate ion channels. The aim of the present study was to examine the role of SGK1 in insulin secreting cells.
**Materials and methods:** SGK1 expression was analysed by quantitative real time RT-PCR and by western blotting in INS-1 cells and isolated rat and mouse islets. Insulin secretion was measured after incubation of INS-1 cells or isolated islets in the presence of test substances using an insulin ELISA kit. Whole cell currents were measured using the standard patch clamp technique. Interaction of SGK1 and Kv channel activity was assessed in *Xenopus* oocytes after injection of mRNA for SGK1 and Kv1.5 by two-electrode voltage-clamp recordings.
**Results:** Under control conditions SGK1 is virtually absent in β-cells. Dex (100 nM) time-dependently increased SGK1 mRNA and SGK1 protein in INS-1 cells and islets. This effect was abolished by 1 µM RU 486. Glucose-induced insulin secretion from dex (100 nM for 4 h) treated INS-1 cells as well as from isolated mouse islets was reduced by 65% and 71%, respectively. In contrast, secretion from islets of SGK1 KO mice was insensitive to dex. $K^+$ channel inhibition by 10 mM TEA and 5 mM 4-AP or by the more selective Kv1.5 channel inhibitor MSD-D, 0.3 and 1 µM, counteracted the effect of dex on secretion in INS-1 cells indicating that increased Kv channel activity may impair glucose-induced insulin secretion after dex treatment. When coexpressed in *Xenopus* oocytes SGK1 upregulates Kv1.5 current pointing to a direct regulation of Kv channels by SGK1.
**Conclusion:** We propose that glucocorticoids impair insulin secretion by inducing SGK1 expression which leads to an increase in Kv channel activity.

## 415

**$K_{ATP}$ channels in beta-cells of pancreatic tissue slices are regulated by physiological ATP concentrations**

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**Background and aims:** Increasing levels of cytosolic ATP following metabolism of glucose inhibit the activity of ATP sensitive $K^+$ channels ($K_{ATP}$ channels). Modulation of the $K_{ATP}$ channel conductance is an important step in the coupling of cell metabolism and excitability leading to insulin secretion. Intracellular ATP concentration of Beta-cells and insulin secreting cell lines was observed to range from 3 to 5 mM at resting conditions and was found to increase for 10–30% when stimulated by glucose. Intracellular ATP concentration is significantly higher than measured IC50 values of ATP for $K_{ATP}$ channel inhibition quantified in inside-out patches, outside-out patches and intact single cells. Modulatory role of ATP/ADP ratio, cytosolic agents like Mg-nucleotides, the long-chain acyl-CoA ester, oleoyl CoA and the membrane phospholipid phosphatidylinositol-4,5-bisphosphate ($PIP_2$) have been suggested to explain the discrepancy. Our group recently established a pancreatic tissue slice preparation and found that even 5 mM ATP dialysis was not sufficient to block the $K_{ATP}$ conductance completely. It is therefore possible to examine the $K_{ATP}$ channel sensitivity to ATP close to physiological conditions. In this study we verified the concentration-dependence of $K_{ATP}$ channel inhibition by ATP in Beta-cells of mouse pancreatic tissue slices in comparison to single cells. Furthermore we tried to gain additional insights into the modulation of ATP sensitivity of $K_{ATP}$ channel in tissue slices.
**Materials and methods:** We studied Beta-cells in mouse pancreatic tissue slices and primary cell culture single cells. Electrophysiological parameters like membrane potential, current and capacitance were measured in standard whole-cell patch-clamp technique. Currents were recorded using a voltage ramp from −150 to +50 mV at a rate of 2 V/s in cells held at −70 mV. Data were acquired at 20 kHz using an EPC10 amplifier with PULSE8.6 software (HEKA Electronic, Germany). Cytosolic $Ca^{2+}$ was measured with a CCD camera (Ixon, Andor Technology, Japan). We applied Fura-2 via the patch pipette to record $Ca^{2+}$ simultaneously with electrophysiological parameters or incubated slices in Fluo-3 to record $Ca^{2+}$ changes in whole islets.
**Results:** Measuring ATP sensitivity of $K_{ATP}$ channels we obtained IC50 values for channel inhibition by ATP of 370 µM for single cells and 860 µM for slices, respectively. Additionally we observed a decrease of the Hill coefficient in the concentration dependence of $K_{ATP}$ channels from 2.3 under conditions were we buffered intracellular $Ca^{2+}$ to 1.1 in unbuffered conditions.Incubating slices in 30 µM acetylcholine we were able to lower the IC50 value in slices to 420 µM ATP which is comparable to the IC50 observed in single cells.
**Conclusion:** Beta-cells in tissue slices are a preparation to link measured $K_{ATP}$ channel sensitivity to appropriate cellular ATP levels. Moreover in this preparation Beta-cells show $Ca^{2+}$-dependent distal signal transduction pathway activity at control conditions which could not be observed in single cells. These results suggest pancreatic tissue slices to represent a stable preparation to study Beta-cells in a state near physiological conditions.

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**Subcellular localisation of endogenous and overexpressed ATP-sensitive $K^+$ channel subunits SUR1 and Kir6.2 in clonal MIN6 β-cells**

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**Background and aims:** ATP-sensitive $K^+$ ($K_{ATP}$) channels play a critical role in regulating plasma membrane potential and thus insulin secretion from pancreatic islet β-cells. However, recent reports have suggested that the $K_{ATP}$ channel subunits Kir6.2 and SUR1 may also be present on intracellular structures, including insulin-containing dense core vesicles and possibly mitochondria. We aimed here to quantify the subcellular distribution and regulation of endogenous $K_{ATP}$ channel subunits in clonal MIN6 β-cells.
**Materials and methods:** MIN6 cells were infected with an adenovirus encoding a dense core vesicle-targeted enhanced green fluorescent protein (phogrin.eGFP) and cultured in Dulbecco's modified Eagle's medium at 25 mM glucose for 12 h. After homogenisation in an isotonic sucrose-based medium, subcellular fractionation was performed using (1) Optiprep™ gradients; (2) vesicle immunoabsorption with an anti-eGFP antibody or (3) fluorescence-activated sorting of individual eGFP-labeled dense core vesicles on a Becton-Dickinson Vantage FACS sorter. Fractions were analysed by immunoblotting using specific antibodies for Kir6.2, SUR1 or organelle markers. Immunocytochemistry or imaging of an over-expressed cyan fluorescent protein (CFP) – Kir6.2 – yellow fluorescent protein (YFP) chimera was performed using a Leica TCS-NT laser scanning confocal microscope or an Olympus IX-70-based TILL photonics fluorescence system.
**Results:** At least 90% of total cellular SUR1 (Mr 140 kDa) and > 30% of Kir6.2 (Mr 45 kDa) immunoreactivity was associated with a dense core vesicle-enriched fraction of clonal MIN6 β-cells, and shown to be present in highly purified preparations of dense core vesicles. Whilst Kir6.2 immunoreactivity was also detected in mitochondrial fractions, SUR1 was undetectable. By contrast, over-expressed YFP/CFP-tagged Kir6.2 was localised either to the plasma membrane or non-vesicular membranes, suggesting a defect in the trafficking of the over-expressed chimera.
**Conclusion:** The majority of Kir6.2.and SUR1 are localised in dense core vesicles in MIN6 β-cells, and may correspond to the "granule-SUR" previously defined as an intracellular target for sulphonylureas. By contrast, the existence of functional $K_{ATP}$ channels on mitochondria would appear to be unlikely in these cells.

## 417

**The role of the imidazoline moiety for the insulinotropic effect of imidazolines**

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**Background and aims:** KU14R is the imidazole analogue of the insulinotropic imidazoline, efaroxan, and has been described to antagonize specifically the stimulation of insulin secretion by imidazoline compounds. However, there is a debate as to whether this compound is acting more as a partial agonist. By comparing the effects of efaroxan and KU14R on stimulus-secretion coupling we sought to clarify whether the imidazoline moiety is essential for the insulinotropic property.
**Materials and methods:** To this end $K_{ATP}$ channel activity, plasma membrane potential, cytosolic calcium concentration and insulin secretion were measured using mouse pancreatic islets or single B-cells therefrom.
**Results:** In the whole cell mode Efaroxan and KU14R achieved a complete inhibition of $K_{ATP}$ channel activity with $IC_{50}$ values of 8,8 μM (Hill slope -1,12) or 31.9 μM (Hill slope -1.54), respectively. When the membrane potential was measured in the whole-cell mode, both compounds were strongly depolarizing at 100 μM, whereas in quasi-intact cells (perforated patch technique), 300 μM KU14R was required for a significant depolarizing effect. Correspondingly, the increase in cytosolic calcium elicited by KU14R was smaller than that by equimolar concentrations of efaroxan, but still discernible. Both compounds (100 μM) did not increase insulin secretion in the presence of 5 mM glucose. However when glucose was raised to 10 mM in the continued presence of the secretagogues, efaroxan produced a strong and biphasic increase in insulin secretion, whereas KU14R produced only a small, monophasic increase lasting less than 10 min. Even at 300 μM the insulinotropic effect of KU14R remained practically negligible.
**Conclusion:** In mouse B-cells, the imidazoline moiety appears not necessary for the $K_{ATP}$ channel-blocking property of this group of compounds but essential for their enhancement of nutrient-induced insulin secretion.

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**The imidazoline, efaroxan, acts as enhancer of nutrient-induced insulin secretion**

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**Background and aims:** The insulinotropic effect of imidazolines was originally ascribed to their ability to block $K_{ATP}$ channels in pancreatic B-cells. Later, an additional effect was described, which appeared to consist in an more effective coupling of exocytosis to increases in the cytosolic calcium concentration ($[Ca^{2+}]i$), i.e. a signal enhancement in the triggering pathway. We now investigated the dependence of the insulinotropic effect of efaroxan on the nutrient supply, i.e. on the amplifiying pathway of insulin secretion.
**Materials and methods:** Insulin secretion, $K_{ATP}$ channel activity and $[Ca^{2+}]i$ were measured using perifused isolated mouse islets and single B-cells.
**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 a strong, biphasic secretion resulted, which was more than fivefold higher than that of controls. At a non-stimulatory concentration of the nutrient secretagogue α-ketoisocaproic acid (KIC, 1 mM in the presence of 1 mM glutamine), the secretion was not enhanced by efaroxan. When KIC was raised to 3 mM, a marked biphasic secretory response could be observed in the presence of efaroxan, while under control condition a weak monophasic response resulted. In contrast, the monophasic secretion elicited by $K^+$ depolarization (in the presence of 5 mM glucose) was only marginally affected (+25%) by efaroxan. Efaroxan at 100 μM blocked $K_{ATP}$ channels in intact B-cells by more than 80% and raised $[Ca^{2+}]i$ in single B-cells in the absence and presence of glucose. In perifused islets 100 μM efaroxan induced large amplitude oscillations of $[Ca^{2+}]i$ in the presence of 5 mM glucose and oscillations superimposed on a sustained $[Ca^{2+}]i$ increase in the presence of 1 mM KIC plus 1 mM glutamine. Raising the nutrient concentration from a non-stimulatory to a slightly stimulatory value did not principally alter these $[Ca^{2+}]i$ patterns.
**Conclusion:** Even though efaroxan blocks $K_{ATP}$ channels and raises $[Ca^{2+}]i$, the relevant effect at 100 μM appears to consist in an enhancement of nutrient-induced secretion, i.e. an interference with the amplifying pathway of insulin secretion.

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**Physiological activation of the glucagon-releasing mouse α-cell by a low glucose concentration involves opening of voltage-dependent L-type $Ca^{2+}$ channels**

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**Background and aims:** Fundamentally different models have been proposed for the signal transduction underlying glucagon secretion. However, until recently the influx of $Ca^{2+}$ causing glucagon secretion from the depolarized α-cell has generally been attributed to the opening of L-type channels, and this is the mechanism underlying glucagon exocytosis in response to cAMP elevation in mouse α-cells. The intriguing observation in mouse islets, that stimulation of glucagon release by a low glucose concentration alone (1 mmol/l) is due to activation of N-type rather than L-type $Ca^{2+}$ channels, raises the possibility that different $Ca^{2+}$ channels may mediate the response to different stimuli. Physiological regulation of glucagon secretion by glucose takes place in the presence of low concentrations of amino acids, many of which stimulate glucagon release. We therefore studied the $Ca^{2+}$ channels involved in the generation of the cytoplasmic $Ca^{2+}$ ($[Ca^{2+}]_i$) response underlying glucagon secretion stimulated by 1 mM glucose in a modified RMPI 1640 medium, which contains a physiological mixture of amino acids.
**Materials and methods:** $[Ca^{2+}]_i$ was measured in dispersed C57BL/6 mouse islet cells with ratiometric fluorescence imaging technique using the indicator fura-2. After each experiment the α-cells were identified by immunostaining.
**Results:** Mouse α-cells exposed to the modified RPMI 1640 medium containing 1 mM glucose exhibited an elevated $[Ca^{2+}]_i$ level with superimpose oscillations or occasionally large amplitude oscillations from the baseline. Raising the glucose concentration to 20 mM inhibited this response in half of the studied cells. The response was immediately abolished by the L-type $Ca^{2+}$ channel blocker nifedipine in all of 11 α-cells, whereas the N-type $Ca^{2+}$ channel blocker ω-conotoxin was inhibitory only in 1 of 6 α-cells.
**Conclusion:** The results indicate that voltage-dependent influx of $Ca^{2+}$ through L-type channels is most important for physiological stimulation of glucagon secretion in response to a low glucose concentration. The occasional inhibition of the $[Ca^{2+}]_i$ response by ω-conotoxin may imply that this influx pathway helps to depolarize the α-cell to open the L-type channels.

# PS 20

# Growth and differentiation of islet cells

## 420

**Isolation and characterisation of a novel population of stem cells from adult pancreas for the generation of beta-cells**

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**Background and aims:** It is known that various postnatal tissues including the brain, muscle and bone-marrow contain multipotent stem cells. The aim of this study was to develop a method for the isolation and expansion of adult pancreatic stem cells (APSC) from mouse and human pancreas and study whether they can give rise to insulin-producing beta-cells.
**Material and methods:** Mouse pancreas and biopsies from human pancreas was digested with collagenase followed by continuous passaging in conditioned medium. After several weeks stable cell lines were analysed for the expression of marker genes and proteins by RT-PCR and FACS analysis. Cell maturation was studied in selected clones by changing culture conditions and addition of defined growth factors.
**Results:** Long-term culturing of pancreatic cells results in the isolation of several murine APSC of whom 3 were selected for further analysis. The clones have a high self-renewal capacity (stable phenotype for more than 2 years) and express different levels of neuroepithelial and beta-cell progenitor cell markers (e.g. nestin, BCRP1, sox1, pdx1, glut2). Under differentiation conditions, mRNA expression of proinsulin was induced indicating that the cells have the ability to develop into beta-cells. The human APSC express a similar pattern of cell markers (nestin, ABCG2, sox1). However, under our culture conditions the human APCS have a lower capacity for in vitro expansion.
**Conclusions:** Our study demonstrates that stem cells are present in adult pancreas which can be differentiated to express insulin. If the methods for cell expansion and ferentiation of human APSC can be improved, then the potential exists to generate autologous beta-cells for cell replacement therapy of diabetes mellitus.

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**Insulin-secreting cells differentiated *in vitro* from expanded human pancreatic islet progenitor cells.**

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**Background and aims:** Cellular replacement therapy is a promising alternative to current treatments for diabetes mellitus but available donor tissue is severely limited. Pancreas-derived endocrine progenitor cells have been suggested as a source for the generation of large amounts of transplantable tissue. However, in vitro differentiation of such cells has resulted only in relatively low amounts of insulin production. We therefore investigated, whether progenitor cells expanded from adult human pancreatic islet preparations in vitro could be differentiated into insulin-producing cells with higher efficiency. Our goal was to achieve a level of insulin production that approaches that of native human pancreatic islets.
**Materials and methods:** The starting material for these studies were isolated human donor islets. We developed a new 3-step cell expansion and differentiation protocol to grow islet derived progenitor cells in vitro and to generate endocrine cell clusters from these expanded cells.
**Results:** Endocrine cell clusters produced in vitro expressed the insulin gene at levels of up to 34% of freshly isolated human islets as judged by quantitative rtPCR (n=6; range of insulin expression 0.7–34%). These clusters secreted C-peptide and insulin in a regulated manner in static incubations in vitro (up to 19% C-peptide secretion compared to fresh islets) and expressed a number of other β cell-specific genes, e.g. PDX1, Beta2/NeuroD, Glut2, Glukokinase, and PC1/3. Like pancreatic islets, they also contained cells expressing the other major islet hormones glucagon, somatostatin, and pancreatic polypeptide, as demonstrated by immunostaining. Expansion cultures of progenitor cells based on the new protocol were not homogeneous but contained at least two phenotypically distinct cell types, one that expresses nestin and vimentin and one positive for the epithelial markers cytokeratin 19 and E-cadherin. The two cell populations possibly interact to allow for efficient differentiation of insulin-producing cells.
**Conclusion:** We demonstrate that highly efficient differentiation of insulin-producing cells from adult human pancreatic progenitor cells in vitro is achievable. Such cells may provide a source of tissue for the cellular replacement therapy of diabetes in the future.

## 422

**Clusterin expression is associated with human islet neogenesis in vitro**

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**Background and aims:** The nature of pancreatic endocrine precursor cells is still unknown. The present study aims to identify molecular markers associated with endocrine precursor cell differentiation. For this purpose, we have studied the expression of several putative markers in a model of human islet neogenesis: Clusterin, which has been associated with pancreas development and regeneration; c-Kit, which has been proposed to represent islet cell precursors; and sialomucin (MUC) and epithelial-specific antigen (ESA) which chararacterize epithelial precursor cells in the breast gland.
**Methods:** Duct-cell enriched human pancreatic cells were cultured in monolayers. The culture period consisted of an expansion phase in serum-containing medium followed by a differentiation phase in serum-free medium and Matrigel overlay resulting in the formation of cultivated human islet buds (CHIBs) after 4–5 week culture. The CHIBs were transplanted under the kidney capsule of nude mice and the grafts were recovered for histological studies after 2 weeks or 3 months. The colocalization of each precursor marker and islet hormones or a universal endocrine cell marker (chromogranin A) was evaluated by double immunofluorescence.
**Results:** We did not identify any c-Kit+/chromogranin A- or MUC-/ESA+ precursor cells during the development of CHIBs. In contrast, the expression of clusterin was regulated in a way consistent with a role in islet development. In the adult human pancreas, clusterin expression was confined to almost all glucagon-producing and part of pancreatic polypeptide-producing cells. No immunoreactivity was seen in the insulin- and somatostatin-producing cells. When the pancreatic cells were cultured in monolayer, clusterin expression still remained in a few pre-existing endocrine alpha and PP cells. At the time of 3-dimentional CHIB formation, clusterin expression increased to a high level and was distributed in almost all of the chromogranin A-positive endocrine cells including alpha, beta, and delta cells. After transplantation, particularly after 3 months, the islet buds became more mature and clusterin expression was more restricted in the alpha cells at the periphery of the islets. Rare PP-positive cells were observed in CHIBs and grafts.
**Conclusions:** The high expression of clusterin in newly-generated as compared with mature islets suggests its involvement in the neogenesis of islets in our culture system.

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**Insulin C-peptide production in cell spheroids generated from umbilical cord blood and bone marrow mesenchymal cells**

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**Background and aims:** In vitro, mesenchymal stem cells (MSC) obtained from umbilical cord blood and from the bone marrow of adult donors can be converted into osteocytes, chondrocytes, adipocytes and other mesenchymal cells, and also into cells with neuronal or hepatocyte characteristics. However, in vitro generation of beta-cells from MSC remains an elusive goal.
**Materials and methods:** Human MSC were obtained from the bone marrow of bones removed during hip replacement surgery, and from human umbilical cord blood. Plastic-adherent cells were propagated and replated in DMEM/10%FCS. Before reaching confluence, the cells were gently scratched from the plastic substrate, and then cultured in non-adherent 6-well plates (Greiner) in serum-supplemented or serum-free culture media. Within one day of suspension culture, the cells formed islet-sized free-floating spheroids. After spheroid formation, media were supplemented with different factors proposed to induce (neuro)endocrine differentiation. Five to seven days later, the spheroids were harvested and studied by immune histology, RT-PCR, and ELISAs for insulin (DRG, Marburg) or insulin C-peptide (Mercodia, Uppsala).

**Results:** Plastic-adherent MSC, or spheroids cultured in standard media containing 10% FCS failed to develop signs for (neuro)endocrine differentiation, and cell homogenates were negative for both insulin and insulin C-peptide. In serum-free media (such as DMEM, TCM-199, or Neurobasal A/B27 medium; Gibco) supplemented with nicotinamide, db-cAMP, and glucagon-like polypeptide, the spheroids well survived, and many cells expressed markers for (neuro)endocrine differentiation (e. g., doublecortin or PGP9.5). Additionally, cell homogenates were consistently positive for insulin C-peptide (up to 10 ng/mg tissue protein), and the presence of insulin- and PDX-1-mRNA could be demonstrated by RT-PCR. Despite the consistent detection of insulin C-peptide (which was proven to be absent in all medium components), tissue insulin levels often were below the detection limit when checked by a human insulin ELISA not cross-reactive with human proinsulin.
**Conclusions:** Production of (pro)insulin-like hormones could be induced in specifically cultured human mesenchymal cells. However, tissue hormone levels were small compared to that of islet cells, and may rather be related to the presence of immature neuroendocrine precursor cells than to that of differentiated beta-cells.

## 424

**Isolation and characterization of SP (side population) cells from neonatal porcine pancreas**

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**Background and aims:** In recent years, a new method for isolation and purification of tissue-specific stem/progenitor cells has been reported based on the differential efflux of Hoechst 33342 dye relative to other cells. These cells have been called the side population (SP) and identified in several tissues and species. Pigs are a good model for the biological study of islets because the properties of insulin secretion from adult porcine pancreatic endocrine cells are very similar to those from human islets rather than mouse islets. In this study, we tried isolation of the pancreatic stem/progenitor cells from neonatal pig by using Hoechst dye and culture and molecular characterization of those cells.
**Materials and methods:** Neonatal (within 3 days old) pigs were sacrificed, and pancreas was removed and digested with liberase. The digest was placed in Histopaque 1077 and pancreatic endocrine cells were collected by density gradient centrifugation. The collected cells were incubated in the presence of the fluorescent DNA-binding dye Hoechst 33342, and cell sorting and analyses were then carried out on flow cytometer. The harvested SP cells exhibiting a high dye efflux activity were plated and cultured on tissue culture plates. To characterize SP and cultured cells, we analyzed the expression of functionally important genes using RT (reverse transcription)-PCR and immunohistochemistry.
**Results:** The SP cells were observed with neonatal porcine pancreas, and sorted on flow cytometer. Some cells in this SP fraction were able to proliferate. The partial cDNA of 24 porcine genes were obtained by homology-based PCR method, and after the sequences were determined, specific pairs of primers and PCR conditions were established for detecting each of the genes by RT-PCR. The cells expressed abcg2/bcrp1 which may be one of the hallmarks for SP cells. The SP cells express both endocrine and exocrine markers as well as several pancreatic hormones, suggesting this population may consist of heterogeneous cell types with various developmental stages. The expression of insulin or glucagon gene has diminished after several days of culture, which may reflect the possibility that the less differentiated progenitors can proliferate and dominate during culture. SP fractions also were mostly pdx-1-negative but half of them were neurogenin 3-positive suggesting commitment to endocrine lineage. The proliferating cells, however, exhibited sustained expression of pdx-1 and neurogenin 3 during culture.
**Conclusion:** We were the first to demonstrate that SP fraction cells do exist in porcine pancreas. Although the population is still heterogeneous, it may be that this method is very useful for enrichment of pancreatic stem/endocrine progenitor cells which have proliferative capacity.
*This study is supported by the Program of Fundamental Studies in Health Sciences of Pharmaceuticals and Medical Devices Agency.*

## 425

**Development of beta cell regeneration drugs by screening for PAX4-inducing small molecules**

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**Background and aims:** Type I diabetic patients as well as late stage type II diabetics suffer from a lack of functional beta cells. As an alternative approach to life-long insulin therapy, we are focusing on the identification of small molecules capable of stimulating beta cell replication or neogenesis in vivo. Pax4 is a transcription factor which is specifically expressed during the early stages of beta cell development and shown to be not only essential for beta cell differentiation but also sufficient to drive the differentiation to insulin producing cells. This has been demonstrated by forced expression of Pax4 in mouse ES cells and ectopic expression of Pax4 during mouse embryonic development. In order to identify small molecule activators of Pax4 expression, we configured a cell-based assay system which is suitable for HTS. In pilot screens we identified pharmacological active compounds which activate Pax4 expression selectively and could therefore be used as starting points to develop beta cell regeneration/replication drugs.
**Materials and methods:** The cellular HTS-compatible Pax4 luciferase reporter gene assay has been based on the human pancreatic duct cell line Capan-1. To discriminate specific from non-specific activators, a counterscreen using a CMV minimal promoter reporter lacking Pax4 promoter elements was employed.
**Results:** We report here key facts of assay development as well as our results from screening a library of about 1300 diverse pharmacologically active compounds. About 50 compounds could be verified to activate Pax4 in a dose-dependent manner without exerting non-specific activity in the counterscreen. Preliminary results indicate that these compounds are also capable of activating the endogenous Pax4 locus. Interestingly, the identified actives cluster into structural and functional classes.
**Conclusion:** The successful identification of Pax4 inducing compounds demonstrates proof of concept for the screening approach and provides us with excellent starting points for further drug development. Moreover, we gained important insights into the nature of signalling molecules and pathways involved in the activation of Pax4. Currently, we are extending our screening approach to diverse compound libraries and continue to validate the active compounds in more complex biological systems to assess their potential to replicate or regenerate beta cells.

## 426

**Interleukin-1β is a physiological regulator of β-cell proliferation and secretory function**

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**Background and aims:** We have shown that chronically elevated glucose concentrations induce β-cell production of the proinflammatory cytokine Interleukin-1β (IL-1β) leading to impaired β-cell function and apoptosis in human pancreatic islets. Furthermore, we demonstrated expression of the Interleukin-1 receptor antagonist (IL-1Ra) in the human β-cell, providing localized protection against IL-1β. In the present study we hypothesize that glucose regulated IL-1β production may also have physiological effect, apparent at low concentrations of IL-1β.
**Materials and methods:** Cultured human pancreatic islets were exposed for 4 days to increasing IL-1β concentrations (0.01 to 10 ng/ml) and analyzed for apoptosis by the TUNEL assay, proliferation by the Ki67 antibody, β-cell function by glucose stimulated insulin secretion (GSIS) and IL-1Ra secretion into the culture medium by ELISA. Intraperitoneal Glucose Tolerance Tests (IPGTT) were performed in IL-1 knockout and wildtype (C57Bl/6) mice after overnight fast.
**Results:** In vitro exposure of islets from nondiabetic organ donors to increasing IL-1β concentrations resulted in a 1.35-, 1.98- and 1.37-fold increase in proliferation by 0.01, 0.02 and 0.2 ng/ml IL-1β ($p<0.05$), but a 1.49 and 1.53-fold decrease in proliferation by 2 and 10 ng/ml IL-1β ($p<0.05$), respectively. This was accompanied by an unchanged number of apoptotic β-cells up to 0.2 ng/ml IL-1β, but a 1.3-, 2.27- and 3.59-fold increase in apoptosis by 1, 2 and 10 ng/ml IL-1β ($p<0.05$), respectively. GSIS and insulin content were significantly induced by 0.02 ng/ml IL-1β and reduced by 2 to 10 ng/ml IL-1β. In parallel, IL-1Ra secretion into the culture medium during the 4-day culture period was increased in islets treated with 0.02 ng/ml IL-1β (3.8-fold increase compared to non-treated control islets), correlating with the concentration of IL-1β leading to the

highest level of β-cell proliferation. To confirm the physiological importance of IL-1β
in vivo, we performed IPGTT in IL-1 knockout and wildtype mice, revealing an impaired fasting glucose and glucose tolerance in the IL-1 knockout mice.
**Conclusion:** These findings indicate a physiological role of IL-1β at low concentrations inducing β-cell production of IL-1Ra, proliferation and improved β-cell function.
*Supported by: Juvenile Diabetes Research Foundation*

## 427

**The proliferation associated protein p8 interacts with a novel splice variant of the DEAD box RNA helicase DDX18 in pancreatic beta-cells**
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**Background and aims:** Previously we have shown that the nuclear protein p8 is expressed in insulin producing pancreatic beta-cells. Glucose regulated expression of p8 is associated with cell proliferation. p8 is also expressed in endocrine pancreatic progenitor cells. To further understand the molecular mechanisms by which p8 promotes pancreatic beta-cell proliferation, the aim of this study was to identify proteins physically interacting with p8 in human pancreatic islet cells.
**Methods and results:** Using a GAL 4-based yeast-two-hybrid system to screen a human pancreas cDNA library we detected a splice variant of the c-myc regulated DEAD box polypeptide 18 (DDX18) as an interaction partner of p8. We confirmed physical interaction between human p8 and DDX18 performing co-immunoprecipitation experiments. The interaction partner of p8 was characterized as a novel splice variant of DDX18 expressed in human pancreatic islets using RT-PCR and sequencing analysis. DEAD box proteins are putative RNA helicases. Specifically DDX18 has been implicated in the regulation of c-myc-dependent cell proliferation. The DDX18-gene is a direct target of c-myc and its expression is upgegulated upon mitogenic stimulation. In Drosophila loss of its homologue protein pitchoune, causes a small size phenotype, resembling the phenotypes of c-myc mutants. This identifies DDX18 as a protein mediating c-myc-dependent growth control.
**Conclusion:** We have identified a novel splice variant of the c-myc regulated DEAD box polypeptide DDX18 as a direct interaction partner of the proliferation associated protein p8 in human pancreatic islets. These results provide a molecular link between p8 and c-myc induced proliferation pathways in insulin producing cells. We propose, that neogenesis of pancreatic beta-cells is in part mediated by the protein p8 through activation of the c-myc regulated protein DDX18.
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## 428

**Adult pancreatic beta cell regeneration and insulin-like growth factors relationship in undernourished rats**
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**Background and aims:** It has been shown that Insulin-like growth factor-1(IGF-1) is expressed in areas of regeneration after partial pancreatectomy (Px), suggesting that IGF-1 plays a role in the growth and differentiation of normal pancreatic tissues. We have previously shown that a 65% protein - calorie food restriction started during the third week of gestation in rats caused reduced beta cell mass at 4 days of life, effect that persisted until adult age. The aim of the present study was to determine whether undernutrition affects the beta-cell growth potential and beta-cell proliferation in the adult undernourished (U) rats and the implication of the IGF system in these processes. To this end, we have used the 90% Px procedure in 8 to 10-week-old U and control (C) male rats and we have investigated before (day 0) and after Px (days 2 and 14): 1) spontaneous beta-cell regeneration and involvement of beta-cell replication, 2) serum IGF-1 and -2 levels and, 3) IGF-1 and -2 gene expression in liver and pancreas.
**Material and methods:** Food restricted rats received 35% of control diet from the last week of gestation until adult period. Spontaneous beta-cell regeneration and beta-cell replication were evaluated by immunocytochemistry and morphometry. Serum concentrations of IGF-1 and -2 were measured by radioimmunoassay and radioreceptor assay respectively. RNAse protection assay was performed to evaluated IGFs expression in pancreas and liver.
**Results:** In both, C and U rats, total beta-cell mass significantly increases as soon as day 2 to reach on day 14 values approximately threefold higher than those of before Px (day 0). By contrast, beta cell replication (BrdU labelling index -BrdU LI-) was significantly higher in C rats on day 0 than in the corresponding U animals. Although the BrDU LI of beta-cell was increased after Px in C and U rats, it was maintained significantly higher in C as compared to U group. IGF-2 was not detected in serum of C and U rats before and after Px. On day 0, both the serum levels of IGF-1 and the liver IGF-1 mRNA expression were significantly reduced in adult U rats as compared to C group. This difference was maintained on days 2 and 14 after Px. Pancreatic IGF-1 mRNA expression was also reduced in U animals on day 0. However, on day 2 after Px, the increase of pancreatic IGF-1 mRNA expression was significantly higher in U group than in control. IGF-2 mRNA gene expression was detected only in the liver and there was no difference between groups of animals before and after Px.
**Conclusions:** Our results show that 1) after Px, beta-cells still have the capacity to regenerate in the adult U rats, with a higher efficiency than controls on day 2, 2) since basal and long-term beta-cell proliferation capacity are impaired in U rats, we suggest that beta-cell neogenesis may play a main role in the regeneration of- beta-cell mass, 3) the increased IGF-1 mRNA pancreatic expression in U rats on day 2 after Px may be instrumental in this process.

# PS 21

# Regulation of pancreatic beta cell mass

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**Pathways to pancreatic cell transformation and β-cell mass expansion**

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**Background and aims:** Diabetes afflicts ~3% of the world population and its incidence continues to rise. Islet transplantation has been proposed as a treatment/cure, though the paucity of donor organs limits its clinical utility. A more efficient solution is to engineer β-cell growth *in vitro* and then transplant the expanded cell mass, or induce β-cell regeneration *in vivo*. The existence/nature of putative human β-cell progenitor cell(s) remains controversial. Most agree that islet regeneration occurs close to ducts and duct-like structures and that ngn-3 and pdx-1 are pro-endocrine genes associated with β-cell neogenesis. Co-expression of these markers in CK-19$^+$ duct cells or dedifferentiated acinar cells suggests that nuclear reprogramming of adult pancreatic cells may represent one possible pathway of islet regeneration, but differentiation of resident stem cells cannot been excluded. To address these questions we used the pancreatic acinar product Islet Neogenesis Associated Protein$^{104-118}$ (INGAP) to expand β-cell mass, both *in vitro* and *in vivo*.
**Materials and methods:** *In Vitro*: Isolated and purified human islets (~90%) or acini (>98% pure) were embedded into a collagen matrix and cultured in DMEM/F12 supplemented with insulin (24 mU/ml), EGF (10ng/ml), dexamethasone (1 μM), 10% FBS [DM] and cholera toxin (100ng/ml). This leads to transformation into duct-like structures (DLS), through cavitation of the inner-cell mass via apoptosis while the remaining coronal cells are transformed into CK19$^+$ cells that lose either their endocrine or exocrine markers. Islet-derived DLSs (I-DLS) were cultured in DM with INGAP (167nM) while acinar-derived DLSs (A-DLS) were cultured with INGAP, gastrin (50nM) + HGF (10ng/ml) [GH] or gastrin + HGF + INGAP [GHI] (n=3). Microarray and qRT-PCR analyses (n=2) were performed on isolated islets, I-DLSs and I-DLS-derived islets. *In Vivo*: Multi-low-dose STZ treated hyperglycemic C57BL6/J mice were administered INGAP (500 μg/day) [I; n=4] or an equivalent volume of saline [S; n=4] for 39 days and sacrificed at 48 days.
**Results:** CK19$^+$/insulin$^-$ I-DLSs overexpress the stemness markers ngn-3, PYY and CD-34 by 3.5-, 5- and 7.5-fold, respectively, compared to islets. INGAP stimulates the redifferentiation of I-DLSs back to an islet-like phenotype with comparable islet specific gene expression. When A-DLSs are treated with INGAP, PDX-1 immunoreactivity increases 23-fold, with no change in insulin reactivity, while GHI treatment stimulates A-DLS-to-islet differentiation [C: 1.7 ± 0.4; GH: 1.3 ± 0.4; I: 2.6 ± 0.8; GHI: 10.9 ± 2.9%; $P<0.05$], similar to the acinar-to-duct-to-islet differentiation observed *in vivo* following glucose infusion and duct ligation. Furthermore, in our *in vivo* model, INGAP administration leads to the reversal of hyperglycemia [S: 23.0 ± 1.5; I: 8.1 ± 1.2 mmol/l; $P<0.01$] through upregulation of PDX-1 in ductal cells [S: 0.77 ± 0.0; I: 19.1 ± 2.2%; $P<0.01$] and increased duct-associated β-cell mass [S: 0.01 ± 0.01; I: 0.27 ± 0.06 mg; $P<0.01$], suggesting the transformation of ductal cells into glucose responsive β-cells.
**Conclusion:** These data support the premise that nuclear reprogramming of DLSs and ductal cells is in fact one pathway for expanding β-cell mass. These data indicate that the adult pancreas is a remarkably plastic organ with cell types retaining the ability to differentiate into duct-like and then endocrine cell types. Exploration of these pathways may lead to new approaches to increase the β-cell mass and reverse diabetes.

## 430

**Cyclin D2 is required for beta-cell replication during postnatal pancreatic development**

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The endocrine pancreas undergoes major remodeling during neonatal development when replication of differentiated beta-cells becomes a major mechanism by which beta-cell mass is regulated. The molecular mechanisms that govern the replication of terminally differentiated beta-cells are unclear. We show that during neonatal development, cyclin D2 expression in the endocrine pancreas coincides with the replication of endocrine cells and a massive increase in islet mass. Using cyclin D2-null mice, we demonstrate that cyclin D2 is required for the replication of endocrine cells but expendable for exocrine and ductal cell replication. As a result, 14 day-old cyclin D2-null mice displayed dramatically smaller islets and a four-fold reduction in beta-cell mass compared to their wild-type littermates. Consistent with these morphological findings, the cyclin D2 null mice had a decrease ability to clear glucose from the blood following intraperitoneal glucose injection. These results suggest that cyclin D2 plays a key role in regulating the transition of beta-cells from quiescence to replication and may provide a target for the development of therapeutic strategies to induce expansion and/or regeneration of beta-cells.
*Supported by: Larry Hillblom Foundation*

## 431

**Respective impact of inheritance and environmental programming on β-cell mass and β-cell function in the GK model**

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**Background and aims:** The Goto-Kakizaki (GK) rat is a spontaneous non-insulin-dependent diabetes model with both defects in β-cell mass and β-cell function. The aim of the present work was to evaluate the respective impact of metabolic environment and genetic factors on these β-cell defects. For this purpose, we performed crosses between Wistar (W) and GK rats. In rats issued from crosses: 1) the β-cell mass was evaluated at 18.5 and 21.5 days of fetal age (E), 2) glucose tolerance and insulin secretion were determined at 4 and 8 weeks (wks) of age.
**Materials and methods:** Rats were issued from crosses between W female and W male (W), W female and GK male (W/GK), GK female and GK male (GK) and GK female and W male (GK/W). Pancreatic β-cell mass was determined by immunohistochemistry and morphometry. Intravenous (iv) glucose tolerance and insulin secretion tests were performed in fasting male rats.
**Results:** β-cell mass was similarly decreased ($p<0.05$) in both W/GK and GK/W as compared to W values (13 ± 2, 11 ± 3, 26 ± 7 μg/pancreas, respectively) and was close to GK values at E18.5 (8 ± 1 μg/pancreas). At E21.5, β-cell mass increased to values close to that in W fetuses in both W/GK and GK/W (195 ± 26, 134 ± 16 and 157 ± 2 μg/pancreas, respectively), although the β-cell mass defect persisted in the GK fetuses (45 ± 2 μg/pancreas). At 4 wks of age, fasting plasma glucose concentration ([PG]) was normal in both W/GK and GK/W as compared to W. In response to iv glucose tolerance test, 5 min after glucose loading, [PG] was increased to the same extent in both W/GK and GK/W as compared to W. At 30 min, [PG] in both W/GK and GK/W were higher ($p<0.05$) than in W, but lower ($p<0.001$) than in GK. Fasting plasma insulin concentration ([PI]) were similar in the four groups of rats. Five min after glucose loading, [PI] reached a peak, lower ($p<0.001$) in both W/GK and GK/W than in W (2.6 ± 0.3, 2.5 ± 0.4 and 6.3 ± 0.8 ng/ml, respectively) and no peak were observed in GK. At 8 wks of age, fasting [PG] in both W/GK and GK/W were higher ($p<0.001$) than in W (1.9 ± 0.1, 1.9 ± 0.1 and 1.3 ± 0.1 g/l, respectively), but lower ($p<0.001$) than in GK (2.4 ± 0.1 g/l). Five min after glucose loading, [PG] increased in both W/GK and GK/W to the same extent that in GK. Thirty min after glucose loading, [PG] were close to GK values in both W/GK and GK/W. Basal [PI] were similar in the four groups of rats. Five min after glucose loading, [PI] reached a peak, lower ($p<0.05$) in W/GK and GK/W than in W (6.0 ± 0.7, 4.1 ± 0.3 and 9.0 ± 1.5 ng/ml, respectively) and again no peak were observed in GK. At 10, 15 and 20 min, the W/GK values were close to W values and the GK/W values were close to GK values.
**Conclusion:** These results suggest: 1) The reduced β-cell mass observed at E18.5 in both W/GK and GK/W is independent of the intrauterine environment. Contrary to GK fetuses, crossed fetuses are able to compensate this defect. 2) Both W/GK and GK/W exhibit a glucose tolerance close to normal and a similarly impaired insulin response to glucose at 4 wks of age. These defects worsen in adulthood and both W/GK and GK/W exhibit a diabetic phenotype at 8 wks of age. 3) At 8 wks old, the insulin secretion in response to glucose was more impaired in GK/W than in W/GK, suggesting a causative role of metabolic environment (intrauterine and/or suckling) on such β-cell functional defect.
In GK diabetic model, primary β-cell mass defect is linked to a genetic determinism, contrary to mature β-cell dysfunction that rather reflects an early programming.

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**p57Kip2 coordinates the differentiation of progenitor cells to beta-cell fate during pancreatic organogenesis**

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We have investigated mechanisms that coordinate the proliferation of pancreatic progenitor cells and their differentiation into endocrine cells during embryogenesis. We show here that cell cycle regulator, p57Kip2, is expressed during a brief period of early pancreatic development in a subset of progenitor cells that have exited the cell cycle. In p57Kip2 null embryos, ectopic BrdU incorporation within pancreatic progenitor cells indicated S-phase reentry of progenitor cells that would have normally exited the cell cycle. Progenitor cells that reentered the cell cycle, however, did not progress through the cell cycle but instead, arrested in G2 phase and were subsequently eliminated by programmed cell death. These perturbations in the progenitor cell population, due to the absence of p57Kip2, led to a decrease in the number of beta-cells generated during organogenesis and thus disrupted the morphology of islets. This study demonstrates that p57Kip2 regulates cell cycle progression and survival of pancreatic progenitor cells to ensure proper allocation of progenitor cells to the beta-cell lineage.

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## 433

**Mice carrying a dominant negative EGF-receptor in the islet cells are hyperglycemic with a reduced β-cell mass**

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**Background and aims:** We have previously shown that epidermal growth factor receptor (EGF-R) signalling is essential for proper pancreatic development. Now we aim to reveal the functional roles of EGF-R family and their ligands in β-cell physiology in the adult. For this purpose, we have generated transgenic mice which carry a mutated dominant-negative EGF-receptor under the pdx-1 promoter.
**Materials and methods:** The pdx1-EGF-R dominant-negative mice (E1-DN) were generated by routine transgenic animal techniques. A C-terminal myc-tag was included for transgene expression studies. Heterozygotes and homozygotes were identified by Southern and dot blot analysis and by PCR. Transgene expression was scored using myc-immunohistochemistry. Blood glucose was monitored from the mice at different ages. Pancreata from newborn and 2 mo old transgenic and wild-type mice were analyzed by immunohistochemichal morphometry (insulin, glucagon, PP, somatostatin). Glucose tolerance was analysed in 4-mo-old animals using intraperitoneal glucose tolerance test (IPGTT; 1 mg glucose/kg), with glucose measurements at 0–120 min.
**Results:** Random blood glucose levels measured at the age of 3–26 wk were consistently slightly but significantly higher in the E1-DN mice than in their wt littermates (10.6 ±;2.5 vs. 9.0 ±;0.9 mmol/l, mean±;SD for n=25 and n=28 mice, $p<0.01$). There was no difference in body weight. Insulin immuno-histochemistry was dramatically different, with large variation of the intensity of insulin staining in the islets of the transgenic mice. Both the proportion of insulin immunoreactive area (0.2% of tissue area vs. 0.6% in the control mice; $p< 0.005$) and the number of islets were reduced (1.3 vs. 1.9 islets/mm$^2$ in the control mice; $p< 0.01$). Interestingly, the E1-DN β-cells were smaller in size suggesting that the cells were relatively inactive in spite of the existing hyperglycemia. There was no change in the number of glucagon producing α-cells. In the IPGTT the fasting blood glucose of the E1-DN mice was normal but their glucose tolerance was impaired (AUC glucose-value 303 mM/min in the E1-DN vs. 501 mM/min in the controls; $p<0.05$).
**Conclusion:** Inactivation of EGF-R signalling in the β-cell impairs the development of a normal β-cell mass. Further studies are ongoing to find out whether this is also associated with a specific defect in β-cell stimulus-secretion coupling.

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## 434

**PDCD4 is a novel gene that is upreglated during pancreatic islet neogenesis, GLP-1 stimulation of whole islets and differentiation of embryonic stem cells to a beta cell phenotype**

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**Background and aims:** Type II diabetes results from a combination of insulin resistance and a decrease in pancreatic function. In the latter stages of the disease, pancreatic endocrine mass decreases and therefore restoration of this mass may provide a therapy to restore pancreatic function. A method to increase islet mass *in vivo* would also be beneficial to type 1 diabetes patients, where islets have been destroyed in an autoimmune reaction. Understanding the process whereby pancreatic islet mass is increased in a murine model might later lead to a non-invasive therapeutic strategy for man. We have developed a method to surgically induce pancreatic duct cell proliferation in the rat, which is followed by an 80% increase in islet mass 56 days after pancreatic stimulation. We subsequently wished to examine which genes were differentially expressed during the process of islet neogenesis and further characterise expression in the pancreas.
**Materials and methods:** Differential display PCR was used to clone gene fragments upregulated during the process of islet neogenesis and the RNAse protection assay used to confirm differential expression. As Programmed Cell Death 4 (PDCD4) was found to be upregulated during islet neogenesis and expression has previously been associated with cell fate in non-pancreatic cells, PDCD4 was characterised in Min6 beta cells, whole rat islets and during differentiation of mouse embryonic stem cells to a beta cell phenotype.
**Results:** An increase in expression of PDCD4 was found after administration of the proliferation- and differentiation-promoting peptide GLP-1 to isolated islets. Although PDCD4 upregulation has been associated with programmed cell death in T cells, the stimulation of apoptosis in Min6 beta cells by hydrogen peroxide results in a decrease in expression. Furthermore, we have found that differentiation of CGR8 embryonic mouse cells to a beta cell phenotype is associated with PDCD4 upregulation.
**Conclusion:** This is the first study showing PDCD4 expression in the pancreas. It is likely that PDCD4 upregulation is associated with proliferation and differentiation in the pancreas and not programmed cell death.

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**Gene expression profile of islet neogenesis in tungstate treated STZ rats**

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**Background and aims:** Throughout the lifetime of mammals, there is a dynamic process of beta cell turnover. There is constantly a low percentage of beta cells that die by apoptosis and some beta cells that are generated from precursor cells of the pancreatic duct. In partial pancreatectomy or glucagon-like peptide 1 treated animals, an increase in pancreas regeneration or neogenesis has been observed. This process is due to an increase in the proliferation and differentiation of precursors cells located in the pancreatic duct towards endocrine cells. Sodium tungstat is an insulin-like agent which decreases hyperglycemia in several animal models of diabetes. Moreover, in STZ and nSTZ rats, tungstate administration increases beta cell mass through neogenesis. The molecular mechanisms controlling pancreas regeneration remain unknown, the objective of this study is to identify genes up-regulated and down-regulated by the treatment using Affymetrix technology.
**Materials and methods:** The STZ-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 group (untreated and treated healthy rats and untreated and treated diabetic rats). The data obtained from the hybridization of the arrays was normalized and analyzed using different softwares such as Affymetrix Data Mining Tool and Microarray Suite, the Bioconductor packages RMA and LIMMA, and SAM. The genes significantly activated or inhibited by the treatment were clustered using dChip and organized with NetAffx Gene Ontology Mining Tool.

**Results:** The list of significant genes differentially expressed due to diabetes, treatment and the combination of both were obtained using the different software packages. Only the genes shown by more than one software program were considered. First of all, the STZ-induced diabetes leads to an alteration of the expression of many genes involved in different pathways in the pancreas. Secondly, tungstate treatment of diabetic rats increases the expression of endocrine (insulin) and exocrine (amylase and dipeptidase) genes; and decreases genes involved in lipid metabolism (HMG-CoA synthase and acetil CoA dehydrogenase). Furthermore, we have observed also an increase several genes involved in proliferation and differentiation processes (TGF-β3 and trefoil factor 2). Finally, no significant differences in gene expression were observed between untreated and treated healthy rats, supporting the data that tungstate does not exert any effect, such as hypoglycemia, in the healthy animals.
**Conclusions:** Tungstate is able to increase the mass of cell beta in diabetic animals through the activation of signaling routes that control the pancreatic plasticity; which, together with tungstate extra-pancreatic effects, leads to the decrease of the glycemia.
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## 436

**A reduction of beta cell mass imposes a secretory strain on the residual beta-cell population in the nicotinamide-streptozotocin Göttingen minipig**

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**Background and aims:** Beta-cell dysfunction and a progressive loss of beta-cell mass are important contributory factors in the development and progression of type 2 diabetes. The aim of this study was to evaluate to what extent a reduction of beta-cell mass can lead to increased stress of the remaining beta-cell population due to increased work-load.
**Materials and methods:** 6 Göttingen minipigs had their beta-cell mass reduced chemically (nicotinamide (NIA; 67 mg/kg) and streptozotocin (STZ; 125 mg/kg)) and 6 were kept untreated. Insulin responses were evaluated both in vivo (during OGTT) and in the isolated perfused pancreas (stimulation with glucose, GLP-1 and arginine) from the same animals.
**Results:** Beta-cell mass, determined stereologically, was reduced by NIA + STZ compared to untreated minipigs (182 ± 76 vs. 464 ± 156 mg; $p < 0.01$). Fasting plasma glucose (4.1 ± 0.5 vs. 3.3 ± 0.3 before NIA+STZ, $p < 0.05$) and $AUC_{glucose}$ after oral glucose (2g/kg) (1383 ± 385 vs. 806 ± 50 mMxmin before NIA+STZ, $p < 0.01$) increased after reduction of beta-cell mass. Insulin responses to oral glucose were higher in spite of the lower beta-cell mass (123 ± 84 vs. 56 ± 24 pMxmin/mg, $p < 0.05$) in NIA+STZ animals.
Insulin responses to glucose, GLP-1 and arginine in vitro showed an even more marked increase per unit of beta-cell mass in NIA+STZ pancreata compared to untreated (83.7 ± 45.9 vs. 34.6 ± 14.4 nmol/mg beta-cells, $p < 0.05$), further demonstrating the increased work performed by the remaining cells in the NIA+STZ pancreata.
**Conclusion:** A primary reduction of beta-cell mass impairs glucose tolerance and leads to an increased work-load of the remaining beta-cells in vivo, which is even more apparent when the pancreas is perfused under controlled conditions. The long term increase in work-load could lead to further dysfunction and/or reduction of beta-cell mass and progression of abnormal glucose tolerance to overt diabetes.

# PS 22

# Beta cell gene expression and transcriptional regulation

## 437

**Modulation of islet gene expression pattern by manipulation of the intra-uterine environment**

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**Background and aims:** We have previously shown that maternal low protein diet (LP) alters the development of the endocrine pancreas in fetal progeny with consequences in adulthood suggestive of intrauterine programming of islet gene expression. Fetal beta-cell proliferation and insulin secretion were decreased while apoptosis and susceptibility to cytokines, Il-1 beta in particular, were increased. These functional effects of LP persisted even after 7 days in culture. Further, we recently demonstrated that LP significantly changed the expression levels of several islet proteins. We have showed that taurine levels in maternal and fetal plasma as well as in fetal islets were decreased by the LP diet. Taurine supplementation to the LP diet restored the functional abnormalities. The aim of the present study was to perform a comprehensive screening of islet gene expression changes in fetal islets induced by the maternal LP diet and to identify the pathways by which taurine is preventive.
**Materials and methods:** Pregnant rats (n=15–20) were fed a LP diet (8% protein) or a control diet (20% protein isocaloric) supplemented or not with 2.5% taurine in the drinking water throughout the gestation period to restore normal taurine levels. At 21.5 days of gestation, fetal pancreatic islet cells were cultured during 7 days in RPMI containing 10% fetal bovine serum. RNA was extracted from batches of 2000 neoformed islets. A total of 5 independent experiments per groups were performed. We used the Gene Chips: Rat Expression 230Aoligonucleotide microarray allowing detection of 15923 genes. Statistical testing for expression differences was performed with Affimetrix Software.
**Results:** Expression of about 9600 genes was demonstrable in fetal islets. Of these, 1112 were significantly down-regulated in the LP group, while 750 were up-regulated when compared to controls (20% protein). Taurine supplementation to LP mothers restored to normal the expression of 1041 (94%) genes that were down-regulated by the LP diet and of 638 (85%) genes that were up-regulated. Normalisation of LP islet gene expression changes involved genes encoding transcription factors participating in pancreatic cell differentiation, growth factors and their receptors, enzymes of glucose metabolism, genes involved in protein trafficking and catabolism as well as in antioxidant defence.
**Conclusion:** Perturbation of the intrauterine environment by LP in rats induces massive modification of gene expression. Taurine supplementation can restore most of the gene expression changes produced by LP. These findings suggest a central role for taurine in islet and beta-cell development and susceptibility to cytokine cytotoxicity
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## 438

**Gene expression profiling of isolated islets of Langerhans treated with glucagon-like peptide-1 (7–36 amide)**

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**Background and aims:** Glucagon-like Peptide 1 (7–36 amide) is a an intestinal hormone secreted in response to nutrient ingestion. Studies have shown that GLP-1 potentiates glucose-stimulated insulin secretion from pancreatic β cells, enhanced insulin gene transcription, increases β cell mass and inhibits apoptosis. Although GLP-1 is inactivated rapidly by dipeptidyl peptidase IV (DDP-IV), synthetic analogs of GLP-1 exist, and efforts have been directed at engineering these peptides so that they are resistant to degradation. It is therefore important to investigate additional actions that GLP-1 may have in pancreatic islets. The aim of the study was to use microarray technology to identify novel genes involved in proliferation and neogenesis that are differentially expressed in islets during incubation with GLP-1.

**Materials and methods:** Freshly isolated islets from C57BL/6 mice were incubated with and without GLP-1 for 30, 60, 90 and 120 minutes (5 replicates of each time point). Total RNA was isolated from the islets and then amplified. 2 µg of aRNA was used to generate cDNA by random priming. cDNA was then labeled with Cy3 and Cy5 mono esters and hybridized onto pancreas specific cDNA microarray chips. These microarray chips were developed by the Beta Cell Biology Consortium (BCBC) and contains 14,688 cDNAs that are expressed in adult and embryonic pancreas. Analysis of the arrays was performed in Acuity software. Normalized intensity values were calculated for each array and the results data was filtered to remove blank and not found spots. The replicates for all the time points were averaged and this average was used for the analysis. K-medians clustering was used to cluster genes with the similarity metric correlation (uncentered) and average linkage clustering in the Acuity program (Axon Instruments). Interesting genes were identified using the GO function classifications from the BCBC annotation web site (http://www.cbil.upenn.edu/EPConDB). The differential expression of interesting genes was verified by quantitative RT-PCR.
**Results:** Over 7000 genes were differentially expressed in response to the incubation of islets with GLP-1. 30% of the differentially expressed genes were unknown. After initial analysis, interesting genes involved in neogenesis that increased in expression over time included Regenerating protein II (regII) and islet neogenesis-associated protein (INGAP). Genes involved in growth and proliferation that were differentially expressed included cadherin I (Cdh1), zinc finger proliferation 1 (zipro1), growth associated protein 43 (Gap43) and hepatocyte growth factor activator (Hgfac). Genes identified which were involved in inhibiting apoptosis were, apoptosis inhibitor 5 (Api5), caspase 9 (casp9) and testis enhanced gene transcript (Vapb).
**Conclusion:** Microarray analysis of differentially expressed genes after treatment with GLP-1 indicated a number of novel known and unknown genes that may be involved in islet proliferation and neogeneisis.

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**Impact of over-expression of carbohydrate response-element binding-protein (ChREBP) on pancreatic beta-cell gene expression profile and insulin secretion.**

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**Background and aims:** The Carbohydrate Response-Element Binding-Protein (ChREBP) is a newly described transcription factor that confers glucose-responsiveness on the expression of glycolytic and lipogenic genes in the liver. Here, we explore the role of ChREBP in β-cell gene expression and insulin secretion.
**Materials and methods:** ChREBP cDNA was cloned from a MIN6 β-cells library and inserted into plasmids pCDNA3 and pAdTRACK-CMV, the latter of which was used to generate an adenovirus using the AdEasy system. Anti-ChREBP antibody was generated in rabbits immunized with ChREBP C-terminal peptide conjugated to Keyhole Limpit haemocyanin. The activity of the liver-type pyruvate kinase (L-PK) promoter was assessed by single cell microinjection of luciferase reporter constructs and photon counting, and mRNA levels by quantitative RT-PCR (TaqMan™) and gene chip microarray analysis. Small interfering (si) RNAs against ChREBP were synthesised with the Silencer siRNA construction kit from Ambion, using primers based on nucleotides 508–527 of mouse ChREBP cDNA, as described. Insulin secretion was assessed by radio-immunoassay.
**Results:** Implicating a role of ChREBP in the up-regulation of the L-PK gene by glucose, microinjection into single MIN6 β-cells of wild-type ChREBP cDNA significantly increased L-PK promoter activity at 30 mM glucose (1.88 ± 0.00472 fold versus 30 mM control), whilst having no significant effect at 3 mM glucose. Conversely, microinjection of an antibody against ChREBP, likely to act as an inhibitor towards endogenous ChREBP, completely reversed the activation by 30 mM glucose of the L-PK promoter in MIN6 cells. Over-expression of ChREBP in primary rat islets infected with ChREBP adenoviruses had no significant effect on glucose-stimulated insulin secretion. Conversely, siRNAs against ChREBP led to 87.32 ± 0.254% decrease in ChREBP protein content in MIN6 cells 48 h after transfection, but had no effect on glucose-stimulated insulin secretion.
**Conclusion:** ChREBP confers glucose-responsiveness to L-PK promoter in pancreatic β-cells, by a mechanism that does not involve changes in glucose-regulated insulin secretion.
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## 440

**Insulin-regulated gene expression in pancreatic beta cells analyzed by oligonucleotide microaarray technique**

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**Background and aims:** Insulin as an anabolic hormone modulates a variety of biological processes and metabolic pathways in insulin target cells. By controlling the amount of numerous proteins at the of level gene expression, insulin affects glucose and lipid metabolism, protein synthesis and degradation, cell growth, differentiation and DNA synthesis. Research over the last 8 years provides convincing evidence that besides the classical insulin target tissues, i.e. liver, muscle and fat, also the insulin-producing beta cell is an insulin target. Therefore we were interested in identifying insulin-regulated genes in the pancreatic beta cell to get a more global understanding of how insulin affects beta cell function.
**Materials and methods:** To determine global transcriptional modifications that are directly mediated by insulin in beta cells, we used the rat Affymetrix U34A array to analyze changes in mRNA levels of 7800 rat genes and expressed sequence tags (ESTs) induced by insulin stimulation of INS1 cells for 5 minutes. RNA profile of the cells was analyzed 60 minutes after insulin stimulation. The short time interval of cell culture after insulin stimulation limits detection to only up-regulated genes because drastic activation of mRNA degradation would be needed to detect down-regulation of gene expression within this time frame.
**Results:** Short-term insulin stimulation leads to the up-regulation of about 2300 genes in INS1 cells. Gene Ontology annotations, RefSeq and PubMed were used to assign the up-regulated genes into functional categories. About half of the genes (45%) correspond to ESTs or genes with unknown functions. Of the genes with known functions, the majority corresponds to genes coding for proteins involved either in intermediary and energy metabolism (19%), in intracellular signaling (16%) or in transcriptional and translation regulation (15%). Other categories consist of genes encoding receptors, carriers and transporters (13%), cytoskeletal proteins (9.5%), proteins involved in vesicle trafficking (6%), in immune response, mitochondrion- and apoptosis related proteins and secreted proteins. Since the beta cell is viewed as an insulin target only recently, the action of insulin on beta cell function in general and gene expression in particular is poorly understood.
**Conclusion:** Our data provide new insight into insulin action on the pancreatic beta cell by identifying novel insulin-regulated genes.

## 441

**Insulin-stimulated c-fos gene transcription in INS1 cells requires signaling via the C-terminal YTHM-motif of insulin receptor B-type, PI3 kinase class Ia and ERK1/2**

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**Background and aims:** Insulin exhibits pleiotropic effects involving mitogenic and/or metabolic events that are tissue- as well as development-dependent. The molecular mechanisms by which insulin exerts selective effects are poorly understood. We have recently shown that one possibility for selective insulin signaling is the utilization of selective signal transduction through the two isoforms of the insulin receptor type A (IR-A) and type B (IR-B). While signaling via IR-A/PI3 kinase Ia/p70s6 k and CaM kinase II up-regulates the transcription of the insulin gene (rIns), the β-cell glucokinase gene (rβGK) requires signaling through IR-B/PI3 kinase C2α-like and possibly PKB/Akt to be up-regulated. In addition to the „metabolic" branch of insulin signaling (activation of PI3K, p70s6 k, PDK1, PKB/Akt, GSK3), insulin also up-regulates the activity of the mitogen-activated protein kinase ERK1/2 in insulin-producing cells. DNA-array microchip analysis of gene expression in response to insulin stimulation in INS1 cells revealed the up-regulation of the proto-oncogene c-fos, which is activated by ERK1/2. The aims of the present study were to analyze, I) which IR isoform contributes to the activation of c-fos transcription by insulin and II) which signaling pathway is involved.
**Materials and methods:** We used c-fos-promoter-driven GFP or DsRed expression as a read-out system to analyze insulin-stimulated c-fos gene transcription in living cells. Application of anti-IGF-IR, anti-IR and anti-IR-B-specific antibodies, as well as transient transfection with FLAG-tagged wild type and mutant IR isoforms were used to analyze which IR isoform and possible binding motif is involved. Pharmacological inhibitor studies of for example PI3K, CaM kinase, MAP kinases, as well as transient transfection studies with the dominant negative form of the PI3K adapter p85 (Δ p85), p85-selective antisense and PDK1-interacting fragment PIF

were performed to evaluate the downstream signaling pathway. The role of IR internalization in c-fos activation was studied by expressing dominant negative dynamin-2 (Dynamin K44A). Simultaneous monitoring of co-expressed c-fos constructs with either rIns-DsRed or rβGK-GFP served as an internal control for the selective signaling pathway via both IR isoforms.
**Results:** Insulin-stimulated c-fos gene transcription I) is dependent on signaling via the IR-B isoform, II) is dependent on the C-terminal YTHM-motif in the IR-B β-subunit, III) requires the activity of PI3 kinase class Ia, and IV) is sensitive to ERK1/2 inhibition by the MEK1 inhibitor PD98059.
**Conclusion:** Insulin-stimulated c-fos gene regulation in the pancreatic β-cell involves signaling via the C-terminal YTHM-motif of the IR-B isoform, PI3 kinase class Ia and ERK1/2.

## 442

**Functional effects of regulated expression of wild type and mutant hepatocyte nuclear factor-1β (HNF1-β) in pancreatic β -cells**

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**Background and aims:** A series of mutations in the transcription factor HNF-1β have been characterised in patients with MODY5, a form of maturity onset diabetes of the young. MODY5 patients display impaired insulin secretory responses to glucose or sulphonylurea and exhibit gradually declining baseline insulin levels. These observations suggest that HNF-1β may regulate β-cell secretory function and control long-term β-cell viability, although its precise role is still unknown.
The aims of this study were to investigate the effects of over-expression of wild type (WT) and mutant forms of HNF1β on the proliferation, viability and secretory function of pancreatic β-cells.
**Materials and methods:** Stably transfected INS-1 cell clones were generated that inducibly express either WT HNF1β or a mutant form identified in MODY5 patients. Two mutants were selected: P328L329fsdelCCTCT (P328del) is a gain of function mutation in which the protein product retains the DNA binding domain of wild type HNF-1β but displays enhanced transactivation potential in reporter assays. A263insGG (A263ins) yields a truncated protein which retains the dimerisation domain and may lead to dominant negative activity.
**Results:** Unexpectedly, induction of WT HNF1β expression resulted in the rapid loss of viability of INS-1 cells. MTS reduction was attenuated within 24 h ($A_{490}$ - uninduced: 0.17 ± 0.01 vs induced: 0.12 ± 0.003; $p<0.001$) and total cell numbers were decreased within 72–96 h (cell count (x $10^5$) at 96 h - uninduced: 8.8 ± 0.38 vs induced: 2.6 ± 0.15; $p<0.001$). This loss of viability was mediated, in part, by increased apoptosis as indicated by enhanced annexin V and caspACE staining of induced cells. Expression of the P329del mutant also significantly inhibited β -cell proliferation (although to a lesser extent than WT HNF1β) but it did not decrease cell viability. The A263ins mutation had no effect on either proliferation or cell viability, even after 96 hours of induction. Flow cytometric analysis revealed that WT HNF1β expression altered the progression of cells through the cell cycle, though this effect was not seen with either mutant. All uninduced INS-1 clones demonstrated a robust insulin secretory response to acute stimulation with a combination of 200 μM IBMX and 0.1 μM PMA or to membrane depolarisation with 30 mM KCl. Induction of WT HNF1β (24 h) caused a marked decline in insulin secretion in response to all stimuli without affecting basal secretion rates (basal uninduced: 0.74 ± 0.08 ng/well; basal induced: 0.92 ± 0.06; IBMX/PMA uninduced: 2.0 ± 0.2; IBMX/PMA induced: 1.2 ± 0.1; $p<0.001$). By contrast, neither mutant altered the insulin secretory response to IBMX/PMA or to KCl.
**Conclusion:** The results suggest that HNF-1β controls the expression of genes that are potentially detrimental to β -cell function. They imply that the expression of HNF-1β must be tightly regulated in order to maintain β -cell viability and secretory function.
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**Activation of NF-kappaB by extracellular matrix in primary rat pancreatic beta cells**

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**Background and aims:** Culture of pancreatic rat primary beta cells on matrix derived from 804G (rat bladder carcinoma) cells and rich in laminin-5 induces their spreading and improves their function and survival. Furthermore this matrix induces the overexpression of IkappaBα, the inhibitor of NF-kappaB, in rat beta cells. The aim of this work was to understand how this matrix induces the overexpression of IkappaBα and to investigate whether the IkappaBα/NFkappaB pathway is involved in the effects of the 804G matrix 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) or on 804G-coated dishes. The cellular localization of NF-kappaB was assessed by immunofluorescence. NF-kappaB transcriptional activity was measured by ELISA (Trans-AM). mRNA and protein levels of IkappaBα and NF-kappaB were assessed by quantitative RT-PCR and by western blot, respectively. Adenoviruses expressing either non-degradable IkappaBα (blocking NF-kappaB nuclear translocation) or constitutive IKKβ (inducing NF-kappaB constitutive activity) were used to investigate involvement of NF-kappaB in the effects of the matrix on the beta cell, with GFP-expressing adenovirus as control. Data are presented as mean±SD for 3 or more independent experiments.
**Results:** The localization of NF-kappaB was clearly nuclear in about 10 % of cells cultured for 1 h on 804G matrix, reflecting possible activation, whereas its localization was mainly cytoplasmic in all cells cultured on pLL (control). NF-kappaB activity was found to be 4.2 ± 1.0 times higher on 804G matrix compared to control ($p<0.05$) after 2 h of culture. IkappaBα protein levels as measured by western blot were decreased after 30 minutes in cells on matrix vs. pLL. This rapid degradation of IkappaBα could activate NFkappaB via the canonical pathway. To begin to investigate the consequences of enhanced NF-kappaB activity in cells on matrix, expression of two of its target genes, IkappaBα and NF-kappaB itself, was evaluated by RT-PCR from 30 minutes to 24 h. The matrix induces rapid overexpression (peak at 4 h) of both IkappaBα (at 4 h: 9.4 ± 2.5 fold vs. pLL; $p<0.02$) and NF-kappaB (at 4 h: 2.5 ± 0.8 fold vs. pLL, $p<0.002$) mRNAs. To assess the function of the enhanced NF-kappaB activity induced by matrix, cells were infected by adenoviruses expressing either non-phosphorylable IkappaBα (IkappaBα n.p, which inhibits NF-kappaB nuclear translocation) or constitutively active IKKβ (IKK c.a., which induces NF-kappaB nuclear translocation). The spreading on matrix of cells infected with IkappaBα n.p-expressing viruses was reduced, while that of cells overexpressing IKKβ was enhanced compared to control, indicating that NF-kappaB activity might be involved in the effects of 804G matrix on cell spreading.
**Conclusion:** These results show that the 804G matrix induces enhanced NF-kappaB activity as well as augmented expression of two known NF-kappaB target genes, IkappaBα and NFkappaB. The enhanced NF-kappaB activity seems to underlie spreading of beta cells on this extracellular matrix. Future work is aimed to establish whether NF-kappaB is also involved in the effects of the matrix on beta cell function and survival.
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## 444

**The protein kinase C inhibitor bisindolylmaleimide can block increased gene expression of the novel Ras GTPase Rhes induced by insulin secretagogues**

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**Background and aims:** Rhes (Ras Homolog Enriched in Striatum) is a novel member of the Ras family of GTPases, identified as a gene regulated by the imidazoline secretagogue efaroxan. Using real-time semi-quantitative RT-PCR, we have examined the expression of Rhes mRNA in islet cells and demonstrate that protein kinase C (PKC) is involved in the up-regulation of Rhes gene expression induced by insulin secretagogues.
**Methods:** Rat islets and RINm5F cells were incubated with efaroxan racemate, efaroxan enantiomers ($10^{-4}$ – $10^{-8}$ mol/l) for 4 h or glibenclamide ($10^{-6}$–$10^{-10}$ mol/l) for 2 h. Enzyme inhibitors were also included. Real-time RT-PCR was performed on mRNA extracted from treated cells.

**Results:** In RINm5F cells, efaroxan caused a dose-dependent increase in Rhes transcript levels (EC50: approx. $5 \times 10^{-6}$ mol/l, increase of 18.2-fold above control with $10^{-4}$ mol/l efaroxan (racemate). Treatment with $10^{-4}$ mol/l (-)-efaroxan, the potent insulinotropic isomer of efaroxan, caused a 39.7-fold (n=3, $p<0.01$) increase in Rhes transcript levels versus the less potent (+)-efaroxan (2.8-fold, n=3). The sulphonylurea glibenclamide also stimulated Rhes gene expression in a dose-dependent manner with an EC50 approx. $10^{-8}$ mol/l, with an increase of 15.6-fold at $10^{-6}$ mol/l glibenclamide. In rat islets, treatment with $10^{-4}$ mol/l (-)-efaroxan resulted in an 8.43-fold increase in Rhes mRNA ($p=0.04$). Co-treatment with the PKC inhibitor bisindolylmaleimide ($5 \times 10^{-7}$ mol/l) caused an 80% decrease in the response to $10^{-4}$ mol/l efaroxan and to $10^{-8}$ mol/l glibenclamide ($p<0.01$ versus secretagogue alone) in each case. Additionally, olomucine ($2.5 \times 10^{-5}$ mol/l, an inhibitor of ERK1/2 MAP kinase) and SB 203580 ($10^{-6}$ mol/l, an inhibitor of p38 MAP kinase) caused a 20% and an 86% decrease, respectively, in the elevated Rhes mRNA levels in response to $10^{-4}$ mol/l (-)-efaroxan. When the two inhibitors were used together, efaroxan-induced increase in Rhes transcript levels was totally abolished. None of the inhibitors affected Rhes gene expression when used alone.
**Conclusion:** The function of Rhes has not yet been clearly identified. Our data identifies Rhes as a novel Ras family member whose gene expression in isolated islets and clonal β-cells is modulated by insulin secretagogues. The induction of Rhes message levels is inhibited, however, by the PKC inhibitor bisindolylmaleimide and involves the activation of ERK1/2 and p38 MAP kinases.
*Supported by: The Wellcome Trust, UK*

# PS 23
# Endocrine regulation of insulin secretion

## 445

**Ghrelin suppresses glucose-induced insulin release via inhibition of $Ca^{2+}$ signalling in rat pancreatic 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 (GH) secretagogue-receptor (GHS-R). We have reported that GHS-R was expressed in the pancreatic islets and that administration of ghrelin influenced insulin release. In this study, we aimed to determine the direct effects of ghrelin on the insulin release in the pancreatic β-cell.
**Materials and methods:** Islets of Langerhans were isolated from Wistar rats aged 8–12 weeks by collagenase digestion. Islets were collected and either used for insulin release experiments or dispersed into single cells. Insulin concentrations were determined using EIA kits. Cytosolic $Ca^{2+}$ concentrations ($[Ca^{2+}]_i$) were measured by dual-wavelength fura-2 microfluorometry.
**Results:** In isolated rat islets, insulin release induced by 8.3 mM glucose was attenuated by 10 nM, but not 0.1 nM, ghrelin, while in 2.8 mM glucose ghrelin had no effect. In rat single β-cells, 10 nM ghrelin markedly suppressed the peak of the first phase $[Ca^{2+}]_i$ responses to 8.3 mM glucose. Ghrelin (10 nM) also attenuated the oscillations of $[Ca^{2+}]_i$ during the second phase responses to 8.3 mM. The attenuation of $[Ca^{2+}]_i$ oscillations by ghrelin was abolished in the presence of GHS-R antagonist [D-Lys$^3$]-GHRP-6 (1 μM). Ghrelin at 10 nM also attenuated the $[Ca^{2+}]_i$ responses to 30 μM tolbutamide, while it had no effect on the $[Ca^{2+}]_i$ responses to 10 μM acetylcholine at 2.8 mM glucose.
**Conclusion:** This study indicates that ghrelin directly reacts with GHS-R on β-cells to suppress glucose-induced insulin release at least partly via inhibition of $Ca^{2+}$ signaling. The inhibition by ghrelin is selective for the actions of glucose and tolbutamide but not acetylcholine. The ability of ghrelin to inhibit insulin release, together with GH-releasing and feeding-stimulating actions, suggests that ghrelin underlies the integrative regulation of energy homeostasis.

## 446

**Protein phosphatase 1 (PP-1) mediated inhibition of insulin secretion by leptin in INS-1 pancreatic beta-cells**
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**Background and aims:** The adipose tissue hormone leptin inhibits insulin secretion and proinsulin gene expression in pancreatic beta-cells. Here we report on the identification of protein phosphatase 1 (PP-1) as a novel leptin regulated gene in pancreatic beta-cells.
**Methods and results:** mRNA from native INS-1 beta-cells and INS-1 cells that were exposed to 6.25 nM recombinant rat leptin for 16 hours was reverse transcribed. Both cDNA pools were subtracted and genes that were differentially expressed upon leptin treatment were identified by PCR. Thereby we identified the PP-1 catalytic subunit Ppp1 ca as a leptin regulated gene in INS-1 cells. The function of PP-1 in insulin producing pancreatic beta-cells was further defined. By RT-PCR and fluorescence immunocytochemistry we find co-expression of PP-1 and insulin in the pancreatic beta-cell line INS-1 and in primary beta-cells from human pancreatic islets. RT-PCR, Northern and Western Blot analysis demonstrated time-dependent inhibition of PP-1 mRNA and protein expression by leptin (100 nM) within 48 h in pancreatic beta-cells. Using a PP-1 specific assay, leptin reduced functional PP-1 enzyme activity by 64% in pancreatic beta-cells, an effect, that could be mimicked by the PP-1 specific inhibitors calyculin A (CalA) and okadaic acid (OA). Leptin, CalA or OA mediated reduction of PP-1 activity lead to profound reduction of intracellular calcium levels and consecutively to both glucose and glucagon induced insulin secretion in pancreatic beta-cells.
**Conclusion:** We identified PP-1 as a leptin regulated gene in pancreatic beta-cells and functionally characterized PP-1 as a key mediator of leptin inhibition of insulin secretion. We conclude, that leptin affects pancreatic

β-cell function at several levels by differential regulation of genes, which may concomitantly contribute to the inhibitory effect on proinsulin biosynthesis and secretion in pancreatic beta-cells. Pathologically enhanced PP-1 activity may contribute to the pathogenesis of hyperinsulinemia in leptin resistant obese patients with diabetes mellitus type 2.

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**26RFa, a novel orexigenic neuropeptide, dose-dependently inhibits insulin secretion in the rat pancreas**

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**Background and Aims:** 26RFa is a novel orexigenic neuropeptide initially isolated from frog brain that is also found in mammalian hypothalamic regions. Primary structures of human, rat and frog 26RFa exhibit 80% identity and the C-terminal octapeptide has been fully conserved from amphibians to mammals, thus suggesting that 26RFa may have important biological functions. The amino acid sequence of 26RFa is similar to that of P518, a peptide whose sequence was predicted from cDNA as the putative ligand of the orphan G-protein-coupled receptor GPR103. GPR103 shares substantial sequence identity with the receptors for neuropeptide Y and galanin, two peptides known to affect insulin secretion. We have investigated the *in vitro* effects of rat-26RFa on insulin, glucagon and somatostatin secretion.
**Materials and Methods:** The study was performed in the isolated rat pancreas perfused *in situ*. Rat 26RFa was synthesized by solid-phase methodology, purified by reversed-phase HPLC and characterized by mass spectrometry.
**Results:** Analytical HPLC showed that the purity of synthetic rat-26RFa was greater than 99%. Infusion of 26RFa, at 100 nmol/l, abolished the insulin response to an increase in perfusate glucose concentration (from 5.5 to 9 mmol/l) (incremental area: 8 ± 8, SEM, vs. 79 ± 7 ng/15 min in controls; $p<0.01$). The inhibitory effect of 26RFa on glucose-induced insulin release was also observed at a lower concentration, 10 nM (incremental area: 33 ± 12 ng/min; $p<0.01$). No effect was found at 1 nM 26RFa (incremental area: 72 ± 4.8 ng/15 min; $p=0.8$). The dose-response curve fitted a sigmoidal curve ($R^2=0.9937$) and the $EC_{50}$ was 7.5 nmol/l. 26RFa failed to significantly modify either glucagon ($F_{15,60}=0.87$; NS) or somatostatin ($F_{15,60}=0.31$; NS) secretion.
**Conclusion:** 26RFa inhibits the insulin response to glucose without affecting glucagon or somatostatin secretion, thus indicating a direct, non-paracrine-mediated effect on the beta cell. In view of its insulinostatic effect 26RFa may be considered a diabetogenic peptide.
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## 448

**Involvement of JAK2 and SrC kinases in GH-regulated $Ca^{2+}$ handling and insulin secretion in BRIN-BD11 cells**

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**Background and aims:** Elevation in cytosolic free $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) is a common mechanism in signaling events to initiate or alter cellular processes. An increased $[Ca^{2+}]_i$ induced by growth hormone (GH) has been observed in relation to different cellular events, including proliferation, metabolism in the pancreatic β-cell. We previously showed that GH-stimulated cell proliferation is associated with a rise in $[Ca^{2+}]_i$ in the pancreatic β-cell. The molecular mechanism underlying the GH effect on $Ca^{2+}$ handling is still unclear. The present study investigated the tyrosine kinases involved in GH-induced rise in $[Ca^{2+}]_i$ and insulin secretion in the insulin-secreting cell line BRIN-BD11.
**Materials and methods:** Measurement of $[Ca^{2+}]_i$ was performed by microfluorometry in cells pre-loaded with Fura-2. Insulin secretion was investigated during batch incubation. Phosphorylation of JAK2 kinase was studied by Western blotting (WB) using anti-phospho-JAK2 and phosphorylation of SrC kinase was performed by immunoprecipitation (IP) of the material with anti-SrC, followed by WB using anti-phospho-tyrosine.
**Results:** GH (500 ng/ml)-stimulated rise in $[Ca^{2+}]_i$ was completely inhibited by the specific JAK2 inhibitor tyrphostin AG490. The inhibitor was also shown an inhibitory effect on $K^+$-induced rise in $[Ca^{2+}]_i$. However, the inhibitor had no longer effect on $K^+$-induced rise in $[Ca^{2+}]_i$ when the intracellular $Ca^{2+}$ pools were exhausted by pretreatment of the cells with thapsingargin. In the presence of the specific SrC inhibitor PP2, GH-induced rise in $[Ca^{2+}]_i$ was partially inhibited. In contrast, its inactive analogue PP3 had no effect. In addition, PP2 did not affect $K^+$-induced rise in $[Ca^{2+}]_i$. GH enhanced insulin secretion by 75% at 3 mM glucose, which was inhibited by the two inhibitors. In contrast, $K^+$-stimulated insulin secretion was not affected by the inhibitors. Stimulation with GH caused tyrosine phosphorylation of JAK2 in the cells with a maximum stimulation at 2 min during 5 min-incubation. The effect of GH on phosphorylation of JAK2 was inhibited by AG490 at the concentration which abolished the effect of GH on $[Ca^{2+}]_i$. In addition, GH stimulated tyrosine phosphorylation of SrC, which was inhibited by the SrC kinase inhibitor PP2.
**Conclusion:** GH activated both JAK2 and SrC kinases in insulin-secreting cells, which mediated the GH-induced rise in $[Ca^{2+}]_i$ and insulin secretion.

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**Low dose ACTH stimulation of cortisol secretion inhibits insulin secretion in healthy males: a possible mechanism for stress-induced glucose intolerance through inhibition of insulin secretion**

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**Background and aims:** It is known that glucocorticoids have diabetogenic effects. Supraphysiological levels of cortisol, as e. g. in Cushing's syndrome, induce gluconeogenesis and impair periferal glucose uptake. Recent studies on transgenic mice with increased glucocorticoid sensitivity in the β-cell due to overexpression have shown that both basal and glucose stimulated insulin secretion is decreased with preserved normal insulin sensitivity. The aim of the present study was to investigate whether also in humans increased cortisol levels supress insulin secretion. Therefore we have investigated the cortisol and insulin response to a low dose ACTH in healthy males with normal glucose tolerance.
**Materials and methods:** 18 healthy males with a normal glucose tolerance test and with a mean age of 52.3 ± 1.2 years, BMI 23.4 ± 0.7 kg /$m^2$ and waist-to-hip ratio 0.87 ± 0.01. 1 microg ACTH (Synacthen) was injected iv 3 hours after breakfast at 10 am. Serum was sampled at 0, +30, +60, +90 minutes for determination of serum cortisol, insulin, C-peptide, and blood glucose.
**Results:** Table show results in mean (SE); *$p<0.005$ vs. 0 min; **$p<0.001$ vs. 0 min. Blood glucose concentrations did not change during ACTH stimulation. Ninety min after ACTH injection mean insulin and C-peptide levels had decreased by 25% ($p<0.001$) and 30% ($p<0.001$), respectively. Peak cortisol levels were reached after 30 min for most subjects and at that time point there were already significant decreases in insulin ($p<0.001$) and C-peptide ($p=0.003$) levels.
**Conclusion:** In humans low dose ACTH, increasing serum cortisol to levels within the upper normal range, causes inhibition of insulin secretion. The inhibitory effect of cortisol on insulin secretion is in line with studies on transgenic animals overexpressing the glucocorticoid receptor in the beta-cell. These findings are compatible with the hypothesis that chronic stress with activated HPA-axis promotes glucose intolerance by inhibition of insulin secretion.

Levels of serum cortisol, insulin, C-peptide, and blood glucose before and after ACTH injection.

| | 0 min | 30 min | 60 min | 90 min |
|---|---|---|---|---|
| Cortisol (nmol/l) | 333 (18) | 533 (21)** | 466 (20)** | 391 (20)** |
| Blood glucose (mmol/l) | 4,74 (0,14) | 4,83 (0,10) | 4,81 (0,09) | 4,83 (0,09) |
| Insulin (pmol/l) | 15 (1) | 13 (1)** | 12 (1)** | 12 (1)** |
| C-peptide (pmol/l) | 0,71 (0,08) | 0,63 (0,07)* | 0,57 (0,07)** | 0,50 (0,05)** |

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**Induction of SGK1 by dexamethasone is accompanied by inhibition of insulin secretion and of proliferation in INS-1 cells**

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**Background and aims:** Mineralocorticoids or glucocorticoids induce the expression of SGK1 (serum and glucocorticoid regulated kinase 1) in most tissue as well as in insulin secreting cells. The kinase has been found to be implicated in the regulation of a variety of ion channels. SGK1, thus, is involved in cell volume regulation, proliferation, apoptosis and cell function. Glucocorticoids are known to inhibit glucose-induced insulin secretion which can finally yield to diabetes mellitus. Since the circumstances which favour glucocorticoid-induced diabetes mellitus are unknown we examined signal transduction pathways which may counteract or amplify SGK1 expression and glucocorticoid-induced inhibition of insulin secretion and proliferation.
**Materials and methods:** INS-1 cells were pretreated in culture with 100 nM dexamethasone (dex) or vehicle for 4 h. Insulin secretion was measured after incubation of the cells for 30 min at 37°C in the presence of test substances using an Insulin ELISA Kit. Cell proliferation was measured by incorporation of BrdU (bromodeoxyuridine labelling for 8 h). Prior to the labelling cells were cultured for 24 h in serum free medium containing 5 mM glucose. Thereafter, exendin-4 (10 nM), IGF (6.6 nM) or forskolin (5 μM) were added 24 h before dex treatment. For Western blotting cells were lysed and subjected to 10% SDS-PAGE. Proteins were blotted onto a nitrocellulose membrane and SGK1 was visualized using a specific primary antibody and a secondary antibody coupled to horseradish peroxidase.
**Results:** Dex induced the expression of SGK1 in INS-1 cells which was significantly higher at 16.7 mM than at 2.8 mM glucose. Ionomycin inhibited dex-induced SGK1 expression at low but not at high glucose. Neither forskolin nor GLP-1 (glucagon-like peptide-1) nor exendin-4, a stable GLP-1 analogue, did influence SGK1 expression. Glucose-induced insulin secretion was inhibited after dex treatment by 68%, an effect reversed by RU 486. Ionomycin supports glucose-induced secretion also after dex treatment. Forskolin but not exendin-4 stimulated secretion was significantly lowered after dex treatment. Inhibition of PI 3 kinase by LY294002 did improve glucose-induced secretion after dex treatment. Proliferation induced by glucose (16.7 mM) or IGF-1 was significantly inhibited by dex, whereas forskolin and exendin-4 increased proliferation was not affected.
**Conclusion:** These results suggest that in the presence of stimulatory glucose concentrations, when the expression of SGK1 is highest, secretion and proliferation are reduced by 68% and 89% respectively. Activation of cAMP signal transduction pathways does not alter SGK1 expression but antagonizes glucocorticoid effects in insulin secreting cells.

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# Signal transduction in islet cells

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**Dual effects of glucose on plasma membrane $PIP_2$ concentration in insulin-secreting cells**

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**Background and aims:** Phosphatidylinositol-4,5-bisphosphate ($PIP_2$) is an important messenger molecule in insulin secretion. Apart from being a substrate for phospholipase C, which catalyzes the formation of inositol-1,4,5-trisphosphate ($IP_3$) and diacylglycerol, $PIP_2$ is known to directly interact with proteins involved in exocytosis. However, little is known about $PIP_2$ dynamics and regulation in pancreatic β-cells. The aim of the present study was to investigate the effects of glucose on plasma membrane $PIP_2$ concentration in individual insulin-secreting cells.
**Materials and methods:** Insulin-secreting MIN6 cells were transfected with the $PIP_2$-binding pleckstrin homology domain from phospholipase C-$\delta_1$ fused to GFP (PH-GFP) and plasma membrane fluorescence was analyzed with evanescent wave microscopy. Co-loading cells with the fluorescent $Ca^{2+}$ indicator fura red allowed parallel recording of the cytoplasmic free $Ca^{2+}$ concentration ($[Ca^{2+}]_i$).
**Results:** Elevation of the glucose concentration from 3 to 11 mM resulted in an immediate rise of plasma membrane PH-GFP fluorescence, indicating that glucose promoted the accumulation of $PIP_2$ in the membrane. This effect was followed within ~2 min by a more or less pronounced decrease to a steady-state fluorescence intensity averaging 2.5 ± 1.0% above base-line. Measurements of $[Ca^{2+}]_i$ with fura red demonstrated that MIN6 cells responded to glucose stimulation with an initial drop of $[Ca^{2+}]_i$, followed by a rise to a sustained elevated level or by regular oscillations resulting from periodic influx of $Ca^{2+}$. Parallel measurements of $PIP_2$ and $[Ca^{2+}]_i$ indicated that the rise and drop of $PIP_2$ coincided with the initial drop and rise of $[Ca^{2+}]_i$, respectively. When $Ca^{2+}$ influx was prevented by hyperpolarization with 250 μM of the $K_{ATP}$ channel opener diazoxide or by removal of extracellular $Ca^{2+}$, elevation of glucose resulted in a sustained increase of membrane $PIP_2$ (7 ± 1% change of fluorescence) that was reversed only when $[Ca^{2+}]_i$ was allowed to increase upon removal of diazoxide or readdition of $Ca^{2+}$.
**Conclusion:** Glucose promotes both formation and $Ca^{2+}$-dependent breakdown of $PIP_2$ in insulin-secreting cells. The dual action of the sugar permits generation of $IP_3$ and diacylglycerol without net loss of $PIP_2$ availability in the plasma membrane.

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**Long-term hyperglycemia induces desensitization of p44/p42 Mitogen-Activated Protein Kinase (ERK1/2) signalling cascade in beta cells, *via* early cellular events**

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**Backgrounds and aims:** The long-term hyperglycemia, characteristic of the diabetic state, contributes to the deterioration of beta cell function, a concept known as beta cell glucotoxicity. In order to understand the molecular mechanism induced by hyperglycemia that underlies the deterioration of the beta cell insulin production and secretion, we investigated whether hyperglycemia impairs the p44/p42 Mitogen-Activated Protein Kinase (ERK1/2) signaling cascade which we have previously shown to be essential for glucose-stimulated insulin secretion and for activation of CREB, a transcription factor crucial for insulin gene transcription and beta cell survival.
**Materials and methods:** We used the MIN6 beta cell line and isolated rat islets. Phosphorylation states of ERK1/2 and CREB were analyzed by western blotting using an anti-phospho-ERK1/2 antibody, which selectively recognizes the doubly phosphorylated, active forms of these kinases and an antibody which detects endogenous levels of CREB specifically when phosphorylated at the $Ser^{133}$ residue. Insulin secretion and content were measured by radioimmunoassay. Morphological nuclear changes were visualized by fluorescent microscopic analysis of cells stained with DAPI.
**Results:** Chronic hyperglycemia (25 mM glucose for 48 h) induced a 50–60% decrease in both basal and 10 mM glucose-stimulated insulin secretion. In beta cells exposed to 25 mM glucose for 48 h, we found that glucose (10 mM, 5 min)-induced ERK1/2 activation was completely abolished. In order to locate the molecular defect(s) responsible for this desensitization mechanism, we used KCl (30 mM, 5 min), a depolarizing agent

which opens the voltage-dependent calcium channel (VDCC) and glibenclamide (100 nM, 5 min), a sulfonylurea which acts as a K-ATP channel ligand. Most interesting, we found that KCl or glibenclamide-induced ERK1/2 phosphorylation remained totally unaffected by chronic hyperglycemia, indicating that the ERK1/2 desensitization is a consequence of a defect located upstream of the K-ATP channel/VDCC system. As a control of the integrity of calcium influxes through the VDCC, nifedipine (2 µM), a VDCC blocker, inhibited KCl-induced ERK1/2 phosphorylation. In high glucose-treated and ERK1/2 desensitized beta cells, there was a marked inhibition of glucose-stimulated CREB phosphorylation. Moreover, the CREB protein expression was significantly decreased by 30% and the expression of insulin and of the anti-apoptotic protein bcl-2, which are both dependent on CREB function, were significantly reduced. As a consequence of the decreased bcl-2 expression, we observed by fluorescent microscopy the emergence of nuclear apoptotic bodies.
**Conclusions:** In this study, we report a novel mechanism, whereby hyperglycemia induces desensitization of glucose-induced ERK1/2 activation which results in impaired CREB function and decreased glucose-stimulated insulin secretion. This desensitization mechanism does not involve a VDCC or a K-ATP channel dysfunction but depends, most probably, on an upstream defect in the glucose metabolism pathway. These data suggest that pharmacological intervention on glucose metabolism in beta cells exposed to hyperglycemia could prevent the deterioration of the ERK1/2 cascade and CREB function, of major importance for the glucose-competent phenotype of the beta cell.

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**Glucose-dependent localization of glucokinase and secretory responsiveness in MIN6 pseudoislets overexpressing a GK-ECFP fusion construct**

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**Background and aims:** The glucose phosphorylating enzyme glucokinase (GK) is the glucose sensor for metabolic stimulus-secretion coupling in pancreatic beta cells. Various studies have provided evidence that post-translational GK regulation takes place in the process of signal transduction. The aim of this study was to investigate the functional relevance of GK regulation for insulin secretion in the mouse MIN6 pseudoislet system.
**Materials and methods:** A GK-fluorescent fusion construct (GK-ECFP) as well as a control fluorescent construct (ECFP) was stably overexpressed in MIN6 cells. Thereafter cells were grown to islet like structures (pseudoislets). GK expression was verified by Western blot analysis and enzyme activity by a photometric assay. Intracellular GK localization was analysed by quantitative epifluorescence microscopy. Glucose-induced insulin secretion was studied by perifusion experiments and radioimmunoassay.
**Results:** Stably GK-ECFP and ECFP transfected MIN6 cells were able to form islet like structures in the same way as observed with non-transfected MIN6 control cells. GK-ECFP MIN6 pseudoislets showed an 14-fold increase in GK activity in comparison to ECFP MIN6 pseudoislets. While in ECFP MIN6 pseudoislets fluorescence was equally distributed across the whole cell, the GK-ECFP construct could be detected exclusively in the cytoplasmic compartment. GK-ECFP MIN6 pseudoislets showed a homogenous cytoplasmic distribution after incubation with 20 mM glucose for 3 h. In contrast incubation at 0 mM glucose resulted in a perinuclear localization of the GK-ECFP fusion protein. GK-ECFP pseudoislets showed an 15-fold increase of insulin secretion after switching glucose concentration from 0 to 20 mM whereas ECFP MIN6 pseudoislets showed only a 8-fold secretory response.
**Conclusion:** These data provide evidence that GK-ECFP transfected MIN6 pseudoislets may serve as an interesting model to study GK regulation and secretory responsiveness within an intact cell. In this experimental system we observed a correlation between GK activity levels, glucose-dependent GK translocation and glucose-induced insulin secretion. The MIN6 pseudoislet system may be useful to evaluate the effects of GK activating compounds upon the physiological regulation of GK in insulin-producing cells.

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**Phospholipase C activity is under tight dynamic control of cytoplasmic $Ca^{2+}$ in insulin-secreting cells**

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**Background and aims:** Phospholipase C (PLC) is involved in the regulation of a variety of cellular processes, including insulin secretion. PLC activity is known to depend on $Ca^{2+}$ and the present study aimed at investigating how PLC activity is regulated by physiological variations of the cytoplasmic $Ca^{2+}$ concentration ($[Ca^{2+}]_i$).
**Materials and methods:** Evanescent wave microscopy was applied to monitor PLC activity in real-time in parallel with $[Ca^{2+}]_i$ using insulin-secreting INS-1 and MIN6 cells transfected with the phosphatidylinositol-4,5-bisphosphate-binding pleckstrin homology domain from phospholipase C-$\delta_1$ fused to GFP (PH-GFP) and loaded with the $Ca^{2+}$ indicator fura red.
**Results:** Elevation of $[Ca^{2+}]_i$ by depolarization with high $[K^+]$ or by closing $K_{ATP}$ channels with tolbutamide resulted in a pronounced loss of PH-GFP fluorescence, demonstrating that elevation of $[Ca^{2+}]_i$ was sufficient to trigger activation of PLC in insulin-secreting cells. Moreover, $[Ca^{2+}]_i$ oscillations resulting from periodic influx of the ion were paralleled by oscillations of PLC activity with a strong positive correlation between $[Ca^{2+}]_i$ amplitude and the degree of PLC activation. To investigate how $[Ca^{2+}]_i$ elevation influenced receptor-mediated PLC activation, cells were stimulated with 100 µM carbachol. In the presence of extracellular $Ca^{2+}$, carbachol induced rapid activation of PLC (27 ± 2% change of fluorescence) followed within ~2 min by partial recovery to a sustained level (12 ± 2%) and complete recovery upon removal of the stimulus. In $Ca^{2+}$-deficient medium containing EGTA, the amplitude of the initial PLC activation was smaller (17 ± 2%) and the sustained PLC activity was almost completely abolished (3 ± 1%). Furthermore, prevention of the carbachol-induced rise of $[Ca^{2+}]_i$ by depletion of intracellular stores with thapsigargin or by loading cells with the $Ca^{2+}$-chelator BAPTA resulted in significant suppression of the carbachol-induced PLC activity.
**Conclusions:** PLC activity in insulin-secreting cells is under tight dynamic control of $[Ca^{2+}]_i$. Elevation of $[Ca^{2+}]_i$ alone is sufficient to activate the enzyme, and $Ca^{2+}$ influx and release from intracellular stores both contribute to enhance receptor-mediated activation of PLC.

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**Glucose stimulates protein kinase A in mouse islets through activation of the $K_{ATP}$ channel-dependent pathway**

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**Background and aims:** The role of cAMP and protein kinase A (PKA) in glucose-stimulated insulin release has not been fully established. The present study therefore aimed at assessing the significance of PKA in glucose triggering of $K^+_{ATP}$ channel-dependent insulin secretion and in glucose amplification of $K^+_{ATP}$ channel-independent insulin secretion.
**Materials and methods:** Insulin release from cultured perifused pancreatic islets of NMRI mice was determined by radioimmunoassay.
**Results:** In islets cultured at 5.5 mmol/l glucose, and then perifused in physiological Krebs-Ringer medium, the PKA inhibitor H89 (10 µmol/l) did not inhibit glucose (16.7 mmol/l)-induced insulin secretion, but inhibited stimulation by the adenylate cyclase activator forskolin (10 µmol/l), suggesting that glucose in this setting does not activate PKA. Forskolin stimulation of insulin secretion was mimicked by the non-selective cAMP analogue 8-CPT-cAMP (250 µmol/l), but not by the Epac selective cAMP analogue 8-CPT-2'-O-Me-cAMP (250 µmol/l), suggesting that forskolin increased insulin secretion through activation of PKA. In the presence of 60 mmol/l $K^+$ and 250 µmol/l diazoxide, on the other hand, which stimulates maximum $Ca^{2+}$ influx independent of $K^+_{ATP}$ channels, H89 (10 µmol/l) inhibited $Ca^{2+}$-evoked insulin secretion by approx. 50% ($P<0.001$), suggesting that $Ca^{2+}$ may activate adenylate cyclase activity. Glucose amplification of $Ca^{2+}$-induced insulin secretion in the presence 60 mmol/l $K^+$ and 250 µmol/l diazoxide was, however, not inhibited by H89 (10 µmol/l) in islets cultured at 5.5 mmol/l glucose, and amounted to a 2.94 ± 0.36(6) fold increase in the absence and a 2.25 ± 0.34(6) fold increase in the presence of H89 (10 µmol/l). After culture at 16.7 mmol/l glucose, glucose (16.7 mmol/l)-induced insulin secretion in physiological Krebs-Ringer medium was augmented and now inhibited by the PKA inhibitor H89 (10 µmol/l), suggesting that culture at 16.7 mmol/l glucose may increase adenylate cyclase activity. In accordance, $Ca^{2+}$-evoked insulin secretion at 60 mmol/l $K^+$ and 250 µmol/l diazoxide was improved. Culture at 16.7 mmol/l glucose did, however, not affect glucose (16.7 mmol/l) amplification of $Ca^{2+}$-induced

insulin secretion in the presence of 60 mmol/l $K^+$ and 250 µmol/l diazoxide, which now amounted to a 2.08 ± 0.40 (3) fold increase during 60 min of incubation.
**Conclusion:** These results suggest that glucose may activate PKA through stimulation of the $K^+_{ATP}$ channel-dependent pathway. Glucose amplification of $K^+_{ATP}$ channel-independent insulin secretion on the other hand occurs by PKA-independent mechanisms.
*Supported by: the Danish Diabetes Association*

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**Low concentration of tacrolimus suppresses glucose-induced insulin secretion from pancreatic islets by decreasing glucokinase activity**
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**Background and aims:** Tacrolimus is widely used for immunosuppressant therapy including various organ transplantations. One of its main side effects is hyperglycemia due to reduced insulin secretion, but the mechanism remains unknown. The reduction of insulin synthesis and content derived from suppressed insulin gene expression found in *in vitro* experiments has been proposed, but several reports suggest that the decrease in insulin release is not necessarily attributable to decreased insulin content. To clarify the mechanism, we have examined the metabolic effects of tacrolimus on insulin secretion at a concentration that does not influence insulin content.
**Materials and methods:** Islets were isolated from Wistar rats by collagenase digestion. The islets were cultured with RPMI medium containing various concentrations of tacrolimus for 24 hours. Insulin secretion was monitored using static incubation and perifusion conditions. The NAD(P)H fluorescence was measured using islets without dye (excitation 360nm, emission 470nm). ATP content was determined by luminometric method. Glucose utilization was determined using [5-$^3$H] glucose. Glucose phosphorylation velocity was measured using islet homogenate.
**Results:** Twenty four hours exposure to tacrolimus (>3 nM) concentration-dependently reduced high glucose (16.7 mM)-induced insulin secretion (1.07 ± 0.04 control vs. 0.87 ± 0.01 ng/islet/30 min, 3 nM tacrolimus, P<0.01). Basal insulin release at 2.8 mM glucose was not affected by tacrolimus. Insulin content of islets was significantly reduced by 10 and 30 nM tacrolimus, but 3 nM tacrolimus had no effect. DNA contents of islets were not affected by 3 nM, 10 nM, or 30 nM tacrolimus. To evaluate the inhibitory effect of tacrolimus on glucose-induced insulin secretion without the affect of insulin content, 3 nM tacrolimus was used. Withdrawal of tacrolimus for 12 hours reversed the suppressive effect of the agent on glucose-induced insulin secretion. In perifusion experiments, the second phase of insulin secretion from tacrolimus-treated islets was suppressed. The average of NAD(P)H fluorescence during a 20 min period after 10 min high-glucose exposure was lower in tacrolimus-treated islets (128.1 ± 1.2 control vs. 122.7 ± 0.7 arbitrary units, tacrolimus, P<0.01). The ATP contents of tacrolimus-treated islets in the presence of 16.7 mM glucose were less than in control islets (9.69 ± 0.99 control vs. 6.52 ± 0.40 pmol/islet, tacrolimus, P<0.01), while in the presence of 2.8 mM glucose there was no difference between the two groups. Glucose utilization in the presence of 16.7 mM glucose was suppressed in tacrolimus-treated islets (103.8 ± 6.9 control vs. 74.4 ± 5.1 pmol/islet/90 min, tacrolimus, P<0.01), while that in the presence of 2.8 mM glucose was not affected by the agent. Glucokinase activity was reduced by tacrolimus treatment (65.3 ± 3.4 control vs. 49.9 ± 2.8 pmol/islet/60 min, tacrolimus, P<0.01), while hexokinase activity was not affected.
**Conclusions:** A low concentration of tacrolimus decreases high glucose-induced insulin secretion from pancreatic islets without affecting insulin content. This inhibitory effect of tacrolimus is due to reduced ATP production and glycolysis derived from decreased glucokinase activity.

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**Insulin-releasing concentrations of D-fructose lower ATP, ADP and ATP/ADP ratio in rat pancreatic islets**
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**Background and aims:** D-fructose (10 mmol/l) augments, in rat pancreatic islets, insulin release evoked by D-glucose (also 10 mmol/l). Even in the absence of D-glucose, higher concentrations of D-fructose (≥ 80 mmol/l) increase insulin output above basal value. We have now investigated whether the insulinotropic action of D-fructose coincides with an increased ATP/ADP ratio in rat islets, as is usually the case with nutrient secretagogues.
**Materials and methods:** Groups of 12 pancreatic islets each, prepared from fed Wistar rats, were incubated for 60 min at 37°C in the presence or absence of D-glucose and/or D-fructose. Islet ATP content was assayed by a luminometric method using an ATP-monitoring reagent containing firefly luciferase and luciferin *(ATP Bioluminescence Assay Kit CLS II, Roche Diagnostic™)*. Islet (ATP + ADP) content was measured by the same procedure after conversion of ADP into ATP by puruvate kinase, in the presence of phosphoenolpyruvate. Islet ADP content was calculated by difference and the paired ratio ATP/ADP was determined.
**Results:** Relative to the basal value found in islets deprived for 60 min of exogenous nutrient, D-fructose (10 and 100 mmol/l) caused a concentration-related decrease ($p < 0.02$ or less) in ATP, ADP and (ATP + ADP) islet content (by about 20–25 and 30–45% at the low and high ketohexose concentration, respectively) and tended to decrease also the ATP/ADP ratio, which averaged, in the presence of 10 and 100 mmol/l D-fructose, respectively, 93.8 ± 5.2% (n = 28) and 86.1 ± 8.0% (n = 29) of the basal value. By contrast, D-glucose (10 mmol/l) increased ($p < 0.005$ or less) the ATP and (ATP + ADP) islet content (by about 70 and 30%, respectively), as well as ATP/ADP ratio (by about 75%). In the presence of D-glucose (10 mmol/l), D-fructose (10 mmol/l) again lowered the ATP and (ATP + ADP) content, as well as ATP/ADP ratio, which averaged, respectively, 75.0 ± 5.1 (n = 30; $p < 0.01$), 82.4 ± 4.6 (n = 30; $p < 0.05$) and 81.8 ± 6.6 (n = 30; $p < 0.05$)% of the mean corresponding value recorded within the same experiment(s) in the sole presence of the aldohexose. The ATP content and ATP/ADP ratio remained, nevertheless, higher ($p<0.02$ or less) in islets exposed to both D-glucose and D-fructose (10 mmol/l each) than in islets deprived of exogenous nutrient.
**Conclusion:** To our knowledge, the present study provides the first example of a dramatic dissociation between changes in ATP content or ATP/ADP ratio and insulin release in pancreatic islets exposed to a nutrient secretagogue. It calls, therefore, for further investigations on the mechanisms by which D-fructose lowers both ATP and ADP islet content and, nevertheless, stimulates insulin secretion.

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**Potentiation of $Ca^{2+}$-induced $Ca^{2+}$ release by cAMP is due to PKA-dependent activation of inositol 1,4,5-trisphosphate receptors in mouse and rat β-cells**
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**Background and aims:** $Ca^{2+}$-induced $Ca^{2+}$ release (CICR) is a phenomenon by which an elevation of the cytoplasmic $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) becomes amplified by release of the ion from the endoplasmic reticulum (ER). Such a mechanism helps to augment $Ca^{2+}$-activated processes like insulin secretion. CICR has classically been attributed to activation of ryanodin receptors (RyRs) but in many types of cells inositol 1,4,5-trisphosphate receptors ($IP_3Rs$) are equally or more important. Different opinions have been expressed regarding the role of RyRs and $IP_3Rs$ in CICR in pancreatic β-cells. It has been claimed that cAMP-mediated activation of RyRs is due to a protein kinase A (PKA) dependent process and/or involves cAMP-regulated guanine nucleotide exchange factor (EPAC). However, other studies have favoured a PKA effect on the $IP_3$ receptors. The present study was undertaken to clarify the type of receptor involved in cAMP-facilitated CICR in primary β-cells and whether the effect is mediated by PKA or EPAC.
**Materials and methods:** $[Ca^{2+}]_i$ was measured with digital imaging technique and the indicator fura-2 in pancreatic β-cells isolated from mice and rats. The cells were exposed to 20 mM glucose to keep the ER loaded with $Ca^{2+}$. Diazoxide was also present to activate ATP-regulated $K^+$ ($K_{ATP}$) channels and clamp the membrane potential at close to the equilibrium potential for $K^+$. The cells were then depolarized by raising the $K^+$ concentration or they were exposed to agents elevating cAMP, cAMP mimetics and antagonists.
**Results:** In the presence of diazoxide $[Ca^{2+}]_i$ remained at basal levels in glucose-stimulated β-cells. $K^+$ depolarization induced a rise of $[Ca^{2+}]_i$ by influx of $Ca^{2+}$. This rise was often accompanied by superimposed $[Ca^{2+}]_i$ transients due to CICR. Elevation of cAMP by exposure to GLP-1, glucagon, forskolin or phosphodiesterase inhibitors induced occasional $[Ca^{2+}]_i$ transients from the base-line in hyperpolarized β-cells and greatly promoted their occurrence in response to depolarization. The $[Ca^{2+}]_i$ transients were also induced by 8-Br-cAMP and the more specific PKA activator Sp-5,6-DCl-cBIMPS but not by the specific EPAC agonists 8-pCPT-2'-O-Me-cAMP or 8-pMeOPT-2'-O-Me-cAMP. Moreover, the transients were partially

suppressed by competitive PKA inhibitors Rp-8-Br-cAMPS and Rp-8-CPT-cAMPS. Ryanodine pretreatment failed to affect the transients but they were abolished by by depleting the ER of $Ca^{2+}$ or by 20 mM caffeine, which inhibits $IP_3$ receptors.
**Conclusion:** The potentiating effect of cAMP on CICR in primary mouse and rat β-cells is due to a PKA-dependent activation of $IP_3Rs$.

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**Effects of sodium tungstate in Min6 beta-cells: potential implications for its antidiabetic action**
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**Background and aims:** The antidiabetic properties of sodium tungstate have been reported in several animal models of type 1 and 2 diabetes. This compound, when administered orally, reduces and, in most cases, normalizes glycaemia without inducing hypoglycaemic episodes. Moreover, we have recently reported an increase in insulinaemia and islet insulin content in neonatal streptozotocin (nSTZ)-induced diabetic rats treated with sodium tungstate for one month. The present work aims to characterise the effect of sodium tungstate on β-cell by studying key components of the insulin transduction cascade as well as its possible role on insulin secretion.
**Materials and methods:** For signalling experiments, cells were incubated (5–60 minutes) with sodium tungstate (0–250 μM) and phosphorylation of MAPK (p42-p44 and p38), Akt and p70 was determined by western blot of whole cell lysates. Immunofluorescence studies were performed to demonstrate the activation and translocation to the nucleus of PDX-1 by tungstate treatment (30 minutes). For secretion assays insulin released was measured by RIA in supernatants of 1 hour static incubations.
**Results:** Sodium tungstate caused a marked increase in p38, p42-p44, Akt and p70S6K phosphorylation in β-cells, confirming the effect of this compound mimicking insulin action. To study whether these kinase phosphorylations could drive a cascade of downstream events we have investigated the putative role of tungstate on PDX-1 activation by immunofluorescence. Our results from experiments with specific inhibitors of p38 (SB202190) and PI3 kinase (LY294002) demonstrated that these proteins are directly involved in the effect of tungstate in PDX-1 stimulation and translocation to the nucleus. Insulin secretion was determined in β-cells where glucose induced insulin secretion was impaired. Interestingly, sodium tungstate treatment recovered insulin release at high glucose concentrations (11.1 mM–16.7 mM; $p<0.05$) reaching the same levels obtained in fully functional MIN6 cells.
**Conclusion:** Sodium tungstate activates insulin signalling pathway in β-cells, resulting in p38 and PI3 kinase dependent PDX-1 translocation to the nucleus. Furthermore, sodium tungstate recovered glucose induced insulin secretion in non glucose responsive β-cells. These events might be involved in the antidiabetic action of this agent.

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**Evidence for insulin resistance of pancreatic α-cells**
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**Background and aims:** Classically peripheral insulin resistance has been well documented. However, if pancreatic α cells are resistant to insulin has not been reported. Our previous publication showed that the expression of pancreatic NPY mRNA was increased and could not be inhibited by insulin treatment over 24 wk in diabetic rats, suggesting that pancreatic NPY produced predominantly by α-cells might be resistant to insulin. To further determine our hypothesis of α cell insulin resistance we conducted the present study.
**Materials and methods:** Wistar rat models with obesity (ob), IGT, and type 2 and type 1 diabetes (T2DM and T1DM) were established with high-fat diet or, high-fat diet/low-dose,or high-dose STZ, respectively. Using these rat models, we observed the distribution of NPY, glucagon (GLC) and insulin(Ins) in islets and the concentrations of NPY, GLC in α-cells and Ins in β-cells with immunofluorescent double-label and single-label techniques and the distribution and concentrations of NPYmRNA expression in islets with in situ hybridization (ISH). The distribution and content of insulin receptor in α cells were also assessed by immunofluorescent double staining with GLC antibody for labeling α cells and insulin receptor antibody for insulin receptor of α cells. Nikon Eclipse 600 SPOT image processing system was applied for quantitative evaluation of the above hormones and insulin receptor.
**Results:** The biochemical and metabolic characteristics of the experimental rats were consistent with the clinical features of related disorders (Ob, IGT and T2DM); The expression of NPY and GLC by α-cells was increased and the distribution of α-cells changed from the sites of periphery to the whole islet; Ins in β-cells and NPY and GLC in α-cells were significantly increased in Ob, IGT and T2DM groups vs. normal control (NC) ($P<0.01$ or $P<0.05$) (Table). The expression of NPY mRNA was not found in islets of normal controls, but distributed in the whole islets of Ob, IGT, T2DM groups (Table).
Insulin receptor protein was identified in α cells in all rats. The amount of insulin receptor was significantly reduced in α cells in T1DM vs NC (16.29 ± 3.59 vs. 24.88 ± 4.11 MOD, $P<0.05$), and also reduced in T2DM, but the difference was not statistically significant (20.34 ± 7.98 vs. 24.88 ± 4.11, MOD).
**Conclusion:** That the elevated expression of GLC and NPY by α-cells was not suppressed by endogenous hyperinsulinism strongly supports the hypothesis of α-cell resistance to endogenous insulin in rat models with various glucose intolerant status; It appears that insulin resistance at the α-cell level do not result from the change of insulin receptor content. Further studies are needed to determine whether this is due to the dysfunction of insulin signaling intracellularlly.

Content of NPY,GLC and Ins of α and β-cells

| | NC | Ob | IGT | T2DM |
|---|---|---|---|---|
| n | 8 | 7 | 7 | 7 |
| Ins | 1425.29 ± 219.78 | 2072.93 ± 339.75** | 1976.33 ± 199.85** | 1708.82 ± 184.39* |
| NPY | 274.92 ± 13.56 | 645.71 ± 121.54** | 598.85 ± 63.37** | 584.97 ± 66.6** |
| GLC | 686.52 ± 126.80 | 1755.15 ± 226.21** | 172.00 ± 107.85** | 1986.24 ± 87.84** |
| NPYmRNA | 0 | 97.55 ± 11.76** | 95.44 ± 18.83** | 94.74 ± 22.41** |

Vs. NC, *P<0.05; **P<0.01

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# PS 25
# Insulin synthesis and secretion

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**Regulation of proinsulin synthesis via the mTOR/S6 Kinase/eIF4E pathway in beta cells**

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**Background and aims:** The regulation of insulin production in the beta cell is a key component in the control of blood glucose and is thought to impact the progression of type 2 diabetes. While much progress has been made in elucidating the mechanisms regulating expression of the preproinsulin gene and secretion of mature insulin, much remains to be learned of how stimulation of the beta cell leads to increased preproinsulin synthesis. Signal transduction pathways play a prominent role in regulating the biosynthetic capacity of a cell, particularly protein synthesis. It has been shown that stimulation of beta cells leads to increased signaling through both the mTOR and MAP kinase pathways and that this may provide part of the mechanism controlling proinsulin synthesis.
**Materials and methods:** To further define components of these pathways that impact proinsulin synthesis, we have undertaken studies in MIN6 insulinoma cells and primary islets. Beta cells underwent metabolic stimulation in the presence or absence of glycolytic, mitochondrial, mTOR, or insulin secretion inhibitors. Cell extracts were subsequently assessed for effects on ATP levels, kinase cascade activation, proinsulin synthesis, and insulin secretion. Additionally, adenovirus-mediated overexpression of S6 kinase and eIF4 was observed for effects on proinsulin synthesis.
**Results:** Glucose stimulation of beta cells leads to activation of mTOR and MAP kinase pathways concomitant with increased proinsulin synthesis and insulin secretion. Inhibition of ATP synthesis (2-DG or FCCP) or treatment with rapamycin led to impaired mTOR pathway activation and impaired proinsulin synthesis, but little or no effect on MAP kinase signaling to eIF4E. These data, in agreement with previous studies by others, suggest that signaling through the mTOR pathway is necessary for metabolic regulation of proinsulin synthesis. Inhibition of insulin secretion with diazoxide did not inhibit glucose stimulation of mTOR pathway activation or proinsulin synthesis, but did preclude MAP kinase activation of eIF4E, suggesting that activation of the MAP kinase pathway via insulin signaling is not required for stimulation of proinsulin synthesis. Adenovirus-mediated overexpression of S6 kinase, an mTOR target, led to augmentation of glucose-dependent proinsulin synthesis, while overexpression of an inactive S6 kinase had no effect. These results suggest that S6 kinase, acting downstream of mTOR, comprises a component in the regulation of proinsulin in the beta cell. Surprisingly, overexpression of wild-type eIF4E led to an inhibition of proinsulin synthesis, suggesting that regulation of insulin synthesis may be distinct from the 5' TOP class of proteins that, like proinsulin, demonstrate stimulation-dependent synthesis. It has been hypothesized that access to eIF4E modulates the specific increase of synthesis of this class of proteins.
**Conclusions:** These data suggest that while components downstream of mTOR are involved in modulation of proinsulin synthesis as seen for other proteins, the regulation of proinsulin synthesis diverges and likely involves mechanisms that are unique. Elucidation of these pathways may provide potential targets for intervention in treating the progression of diabetes.
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**Inhibition of calpains blocks rat pancreatic beta cell spreading and insulin secretion**

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**Background and aims:** In addition to promoting insulin secretion, an increase in cytosolic $Ca^{2+}$ has been shown to be crucial for the glucose-induced spreading of beta-cells on an extracellular matrix. Calpains are $Ca^{2+}$ dependant cysteine proteases, which are involved in a diverse spectrum of cellular responses, including cytoskeletal rearrangements and vesicular trafficking. This work aims to assess whether calpains may also be implicated in the process of $Ca^{2+}$ induced insulin secretion and spreading of rat beta cells.
**Materials and methods:** All experiments were performed with FACS-sorted rat pancreatic beta cells plated either on poly-L-lysine (PLL) or on a matrix produced by a rat bladder carcinoma cell line (804G matrix). Cells were incubated for 24 h under control conditions or in the presence of 3 different inhibitors of calpain: N-Ac-Leu-Leu-norleucinal (ALLN, 200 µmol/l), calpeptin (35 µg/ml) and EST ((ethyl (+)-(2S,3S)-3-[(S)-3-Methyl-1-(3-methylbutylcarbamoyl)butyl-carbamoyl]-2-oxiranecarboxylate, 200 µmol/l). Cells were analyzed for attachment and spreading by microscopy, and insulin secretion was measured after a static incubation test (1 h basal + 1 h stimulated) by radiommunoassay. Data are presented as mean±SEM for 3 or more independent experiments.
**Results:** ALLN, EST and calpeptin largely inhibited cell spreading on 804G, whereas cell adhesion was not significantly modified. Spreading did not occur when beta cells were cultured on PLL, but treatment with calpain inhibitors did not affect the adhesion and the morphology of cells attached on PLL. ALLN, EST and calpeptin inhibited insulin secretory responses to 16.7 mM glucose from beta cells attached onto 804G matrix by 83% ± 3.4 ($P$= 0.001), 60.4%± 1.15 ($P$=0.04) and 62.3% ± 10.4 ($P$=0.026) respectively, compared with cells in control conditions. Removal of the inhibitor from the cells restored spreading and insulin secretory response after 24 hours. Treatment of cells attached on PLL with calpain inhibitors resulted also in a significant ($P$=0.035) decrease in the response to 16.7 mM glucose. In addition, incubation with calpeptin also attenuates insulin secretory responses to nonglucose secretagogues including isobutylmethylxanthine, phorbol 12-myristate 13-acetate and KCl.
**Conclusion:** These results clearly indicate that inhibitors of calpain inhibit both insulin secretion and spreading of beta cells. Therefore it is suggested that calpain could be a mediator of $Ca^{2+}$-induced-insulin secretion and beta cell spreading.
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**Re-evaluation of the biphasic pattern of glucose-stimulated insulin secretion in the mouse uncovers a clear ascending second phase**

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**Background and aims:** Hyperglycaemic clamps in humans have established a glucose-stimulated insulin secretion (GSIS) pattern characterized by a rapid 1st phase and an ascending 2nd phase. An ascending 2nd phase is also observed in the perfused rat pancreas and in perifused rat islets. However, in the perfused mouse pancreas and in perifused mouse islets, a rapid 1st phase is followed by a flat 2nd phase. This has raised concern on the extrapolation of insulin secretion data from the mouse to the human species. We have therefore re-evaluated the pattern of GSIS in mice by using a newly developed hyperglycaemic clamp technique in vivo, in combination with perifusions of isolated islets in vitro.
**Materials and methods:** In the first experimental series, anaesthetized female C57BL/6J mice fasted for 4 h were cannulated in the jugular vein and carotid artery. After obtaining a baseline arterial sample, a bolus of G (0–0.75 g/kg) was rapidly injected iv, followed by G infusion (20–40 g/dl) at a variable rate to clamp circulating G at 11.1, 16.7 or 30 mmol/l, or remaining at 8.5 mmol/l for 90 min. Blood G was measured every 5 min. Samples for insulin assay were taken at 1, 5, 20, 50, 70 and 90 min. In the second experimental series, isolated islets from C57BL/6J mice were cultured overnight in RPMI medium (8.5 mmol/l G) and then perifused with Krebs medium for 180 min. G was 8.5 mmol/l throughout or was raised to 11.1, 16.7 or 30 mmol/l after 40 min. One series started in 3 mmol/l G. Insulin was measured in the effluent fractions collected at 0.7–3 min intervals.
**Results:** Baseline G was 8.7 ± 0.2 mmol/l. During the clamps, mean G was 8.9 ± 0.2, 11.2 ± 0.3, 16.8 ± 0.6 and 30.8 ± 1.5 mmol/l, respectively. The rapid rise of G elicited a clear 1st phase insulin secretion which peaked at 1 min at all hyperglycaemic levels. After a nadir at 5 min, an ascending 2nd phase of similar magnitude (4-fold higher than baseline levels, P<0.001) occurred at 16.7 and 30 mmol/l G. However, raising G to 11.1 mmol/l did not elicit a 2nd phase. In perifused islets, a concentration-dependent 1st phase of insulin secretion peaked at about 3 min. It was followed, after a nadir at 8–10 min, by a distinct 2nd phase that was 2.5, 8 and 13-fold above the 8.5 mmol/l G baseline at 11.1, 16.7 and 30 mmol/l, respectively. This 2nd phase was flat in 11 mmol/l G and clearly ascending at higher G. However, when G was raised from 3 mmol/l to 16.7 mmol/l (a traditional protocol), 1st phase insulin secretion was 2-fold larger, but 2nd phase was flat and 1.3-fold smaller than after raising G from 8.3 to 16.7 mmol/l.
**Conclusion:** A rapid 1st phase and an ascending 2nd phase of GSIS are seen in mice during a hyperglycaemic clamp in vivo when G is rapidly raised from 8.3 to 16.7 or 30 mmol/l. An ascending 2nd phase of insulin secretion is

seen also in mouse islets perifused in vitro when the G concentration is increased to 16.7 or 30 mmol/l G from the physiological concentration of 8.5 mmol/l, whereas the 2nd phase is flat when the prestimulatory G has been lowered. Therefore, GSIS shows an ascending 2nd phase in the mouse, as in other species, when a physiological protocol is used. An influence of the prestimulatory conditions must be taken into account in the interpretation and quantification of the two phases of GSIS.

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**Protein acylation and the second phase of insulin secretion**

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**Background and aims:** Studies using cerulenin, a known inhibitor of protein acylation, suggest that protein acylation plays a critical role in the second phase of glucose-stimulated insulin release. Our study was designed to evaluate the importance of protein acylation in a highly glucose-responsive β-cell line, the INS 832/13 cells which manifest the $K_{ATP}$ channel-independent (or amplification) pathway.
**Materials and methods:** Protein acylation was determined by incubating the cells with $^3$H-palmitic acid in the absence and presence of stimuli, followed by chloroform/methanol extraction (to remove palmitate incorporated into lipids) and subsequent protein precipitation on filter membranes. The amount of acylated protein was detected by liquid scintillation counting. Insulin release was measured by RIA. Nutrient metabolism was measured as $O_2$ consumption by Clarke electrode, and by $^{14}CO_2$ and $^3H_2O$ production from radiolabeled glucose.
**Results:** We studied the effects of glucose and BCH (a non-hydrolysable analog of leucine). BCH stimulates insulin release without changing palmitate oxidation or its incorporation into lipids and thereby eliminates concerns about altering the specific activity of the radioactive tracer. In response to 16.7 mM glucose or 20 mM BCH we found an increase of 95 and 90% respectively in the incorporation of $^3$H-palmitate into protein. These increases were inhibited concentration-dependently using the acylation inhibitor cerulenin (30 μM cerulenin: 75% inhibition; 100 μM cerulenin: 90% inhibition vs 20 mM BCH). Cerulenin also strongly decreased the incorporation of $^3$H-palmitate under basal (2.8 mM glucose) conditions. This led us to evaluate the effects of cerulenin on some metabolic parameters in the INS 832/13 cell line and to reevaluate them in rat pancreatic islets. We found a powerful and concentration-dependent inhibition of glucose oxidation in the INS 832/13 cells (over 70% inhibition at 2.8 and 16.7 mM glucose with 100 μM cerulenin). Measuring oxygen consumption and glucose utilization and oxidation rates in rat pancreatic islets, we confirmed the strong inhibitory effect of cerulenin on glucose and mitochondrial metabolism. Cerulenin treatment in the INS 832/13 cells resulted in a stimulation of insulin release (in contrast to rat islets) at concentrations of 100 and 300 μM cerulenin under basal conditions (even in the absence of $Ca^{2+}$) and when either BCH or KCl were used as stimuli. Glucose-stimulated secretion however was inhibited at high concentrations. It has been reported that cerulenin treatment also results in inhibition of fatty acid synthase (FAS) an enzyme which, in contrast to pancreatic islets, is present in clonal β-cells at appreciable levels. We speculated that the paradoxical stimulatory effect on secretion (despite the inhibition of metabolism) might result from inhibition of FAS and studied the effects of C75 (an inhibitor of FAS) on insulin release in INS 832/13 cells. C75 mimicked the stimulatory effect of cerulenin on secretion under both $Ca^{2+}$-containing and $Ca^{2+}$-free conditions.
**Conclusion:** Stimulation of INS 832/13 cells with glucose or BCH is associated with increased incorporation of palmitate into protein and provides evidence in favor of a role for protein acylation in nutrient stimulated insulin release. However, the strong inhibitory effect of cerulenin on metabolism and possible additional actions on targets like FAS resulting in perturbation of lipid pathways in clonal β-cells, makes it unsuitable to evaluate the effects of protein acylation in β-cells.

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**In cultured beta cells PED overexpression alters intracellular calcium levels thereby impairing glucose induced insulin secretion**

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**Background and aims:** Both insulin resistance and impaired insulin secretion contribute to the progression from normal glucose tolerance to overt type 2 diabetes. These two defects are genetically determined, but the genes responsible for them have not been elucidated yet. Previously, we have shown that the protein PED has an important role in controlling glucose metabolism *in vitro*. In transgenic animals (Tg), PED overexpression causes peripheral insulin-resistance and reduced glucose tolerance. PED Tg also exhibit subnormal insulin responses to hyperglycemia, indicating that beta-cell dysfunction may contribute to impairing glucose metabolism in PEDTg. However, the physiological role of PED in beta-cell is still unknown. In the present work we have investigated the function of PED in cultured beta-cells and the significance of PED overexpression to insulin secretion.
**Materials and methods:** To this aim, we have used the glucose-responsive MIN-6 beta-cell line. We measured insulin secretion by ELISA assay and used Fura-2/AM as an indicator of intracellular calcium concentration.
**Results:** Exposure of these cells to 20 mM glucose or to 4 μM gliburyde induced 5- and 4-fold stimulation of insulin secretion, respectively. The overexpression of PED in MIN-6 beta-cells determined a 90% decline of insulin secretion induced by both glucose and sulfonylurea stimuli. Transfection of a specific PED antisense oligonucleotide reduced the expression of endogenous PED by more then 80% in wild-type MIN-6 cells (MIN-C) and enhanced glucose-induced insulin secretion by 2.5-fold. This same antisense almost completely rescued glucose responsivity in PED-overexpressing beta-cells (MIN-P). Glucose-elicited insulin secretion is completely dependent on $Ca^{2+}$ entry into the cytosol through $Ca^{2+}$ channels. The subsequent increase in intracellular $Ca^{2+}$ then activates insulin exocytosis. Most likely, a number of protein kinases, including the calcium and calmodulin-dependent protein kinase (CaMK) as well as protein kinase C (PKC), are involved in these distal steps. Based on Fura-2/AM analysis, intracellular $Ca^{2+}$ concentrations in the MIN-C cells showed a 2-fold increase upon 20 mM glucose exposure. By contrast, in MIN-P cells $Ca^{2+}$ levels were constitutively increased as compared with control cells and no further induced by glucose stimulation. Also, in PED overexpressing beta-cells glucose-induced activation of the calcium-dependent enzymes CaMKII and PKC was reduced by 80 and 60%, respectively, as compared to the wild-type beta-cells.
**Conclusion:** PED overexpression in beta-cell impairs glucose-induced insulin secretion by altering $Ca^{2+}$ intracellular levels and dysregulating the activity of $Ca^{2+}$-dependent kinases.

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**Homocysteine, a metabolic syndrome risk factor, exerts detrimental effects on insulin secretion and pancreatic beta cell function**

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**Background and aims:** Elevated homocysteine (homocysteinemia) as observed in type 2 diabetes is widely regarded as an important risk factor for development and progression of the metabolic syndrome. However, the mechanisms underlying the association between homocysteine and development of diseases of the metabolic syndrome, including type 2 diabetes, remain unclear. Although homocysteine is associated with reduced insulin sensitivity, the contribution of elevated levels of this amino thiol to the dysfunction and demise of insulin secretion in type 2 diabetes has not been determined. As such, the aim of this study was to examine the effects of elevated concentrations of homocysteine on insulin secretion and key beta cell signalling pathways.
**Materials and methods:** Insulin release (mean ± SEM) from clonal glucose-responsive pancreatic BRIN-BD11 beta cells was measured during acute 20 min incubations (n=6).
**Results:** Incubation with 100–250 μmol/l homocysteine resulted in a dose-dependent 30–60% decrease ($p<0.001$) in basal insulin secretion at 1.1 mmol/l glucose ($0.98 \pm 0.04$ ng/$10^6$ cells/20 min). In the presence of stimulatory glucose (16.7 mmol/l; $2.49 \pm 0.16$ ng/$10^6$ cells/20 min), 50–250 μmol/l homocysteine caused a marked progressive 37–75% decrease ($p<0.001$) in insulin secretion compared to 16.7 mmol/l glucose alone. Similarly, at stimulatory glucose, incubation with 50–100 μmol/l homocysteine significantly reduced the insulin secretory effects of nutrient secretagogues 20 mM alanine (by 36%, $p<0.001$), 20 mM arginine (by 32–35%, $p<0.01$) and 20 mM ketoisocaproic acid (by 36–56%, $p<0.01$). Homocysteine (50–100 μmol/l) reduced the insulinotropic actions of 10 nmol/l of incretin hormones, glucagon like peptide-1 (7–36) amide, gastric inhibitory polypeptide and cholecystokinin-8 by 39–53% ($p<0.01$-$p<0.001$), 50–51% ($p<0.001$) and 34–36% ($p<0.001$), respectively. However, equimolar homocysteine did not influence the insulin secretory responses to 25 μmol/l forskolin or 10 nM phorbol 12-myristate 13-acetate. Interestingly, acute (20 min) exposure to 50–100 μmol/l homocysteine exerted no effect on cellular viability, insulin content, or gene expression of insulin, GLUT2, glucokinase, voltage-dependent $Ca^{2+}$ channel or the two component beta cell $K_{ATP}$ channel (Kir6.2, SUR1).

**Conclusion:** Acute exposure to concentrations of homocysteine which have been observed in hyperhomocysteinemia, results in a marked deterioration of glucose-induced insulin secretion and inhibits the stimulatory actions of a number of key insulin secretagogues. These data suggest that homocysteine, a risk factor for the metabolic syndrome, may play a role in the progressive dysfunction and demise of insulin secretion and pancreatic beta cell function in type 2 diabetes.

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**Urotensin-II is present in pancreatic extracts and inhibits insulin secretion**

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**Background and aims:** Preliminary studies have shown that urotensin-II (U-II), a vasoactive peptide, inhibits insulin secretion in the rat pancreas. Recently, an octapeptide sharing the C-terminal U-II amino acid region (urotensin-related peptide, URP) which activates U-II receptors, has been isolated from rat brain extracts. We have investigated: 1) the presence of U-II-immunoreactivity in pancreatic extracts and 2) the effects of U-II and structural analogs on insulin secretion.
**Materials and methods:** The study was performed in the perfused rat pancreas. Rat U-II, URP, $Ala^8$-U-II $_{(4-11)}$ and 3-iodo-$Tyr^6$-U-II $_{(4-11)}$ were synthesized by solid-phase methodology. In pancreatic extracts, U-II-like immunoreactivity was quantified by RIA and its molecular forms were characterized by HPLC.
**Results:** U-II-immunoreactivity was found in rat pancreatic extracts (288 ± 78 pg/pancreas) and the HPLC profile showed the presence of native U-II. In the perfused rat pancreas, U-II inhibited glucose-induced insulin release in a dose-dependent manner (sigmoidal curve; $R^2$=0.9851; $EC_{50}$=1.2 nmol/l). U-II also reduced the insulin response to BayK8644, an L-type calcium channel agonist (incremental area: 17 ± 2 vs. 39 ± 6 ng/5 min in controls; $p<0.05$). URP and 3-iodo-$Tyr^6$-U-II $_{(4-11)}$, two peptides possessing the U-II C-terminal cyclic hexapeptide, also inhibited glucose-induced insulin release (12 ± 4 and 19 ± 4 ng/15 min, respectively, vs. 43 ± 7 ng/15 min in controls; $p<0.05$). Substitution of the C-terminal Val by an Ala residue ($Ala^8$-U-II $_{(4-11)}$) led to an inactive peptide. It must be mentioned that none of the compounds tested affected the pressure of the perfusion system.
**Conclusion:** 1) The presence of U-II in pancreatic extracts as well as its insulinostatic effect suggest its implication in the control of B-cell secretion. 2) Structure-function studies demonstrate the importance of the C-terminal region of the U-II molecule, the most conserved throughout different species, to exert its biological activity.
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**Liver X receptor activation modulates insulin secretion in pancreatic β-cells**

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**Background and aims:** Development of type 2 diabetes is associated with increased levels of cholesterol. Liver X receptors (LXRs), transcription factors of a nuclear hormone receptor family, control cholesterol levels through regulation of reverse cholesterol flux. Recently, it has been shown that LXRs play a role in the control of glucose homeostasis. Activation of LXRs increases insulin sensitivity and thereby decreases blood glucose in diabetic animals. The objective of the study was to examine expression and effects of LXR activation in pancreatic β-cells.
**Materials and methods:** Insulin secretion and insulin content were measured from isolated rat pancreatic islets and insulinoma MIN6 cells. Gene expression was examined with RT-PCR and immunoblotting.
**Results:** LXR α and β are expressed in pancreatic islets as well as glucagon-secreting and insulin-secreting cells. Culture of islets or MIN6 cells with the synthase and acetyl-CoA carboxylase. LXR activation also produced an increase in glucokinase protein and pyruvate carboxylase (PC) activity levels. The PC inhibitor, phenylacetic acid, abolished the increase in insulin secretion in cells treated with T0901317.
**Conclusions:** The results suggest that LXRs control insulin secretion and biosynthesis via regulation of glucose and lipid metabolism in pancreatic β-cells.

## 469

**Heterogeneity of the glycemic response to OGTT in mitochondrial diabetes. A study of maternally inherited diabetes and deafness (MIDD)**

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**Background and aim:** There is an increasing focus on mitochondrial diabetes and the most common type is caused by the point mutation A3243G in the tRNA, Leu (UUR) gene which may lead to Maternally Inherited Diabetes and Deafness (MIDD).
The pathophysiological mechanisms leading to mitochondrial diabetes and MIDD are complex, but the oxidative phosphorylation in the respiratory chain is essential to the glucose-induced insulin secretion, and impairment may cause a progressive insulin secretion defect. Intracellular co-existence between mitochondria with mutant (mtDNA A3243G) and wild-type genome, (heteroplasmicity), exist and individual degree of heteroplasmicity (DH) contribute to the difference in diabetic phenotypes.
The aim is to evaluate the glycemic response to an OGTT of mtDNA A3243G mutation positive subjects with and without known diabetes.
**Material and methods:** 21 individuals from four pedigrees were investigated: (Mean, range) Age: 45.6 years (18–74), BMI: 22.0 (18.5–27). Male:female-ratio 7:14.
Prior to the study eight of the subjects were diagnosed with diabetes mellitus (DM): (one treated with diet, one treated with oral antidiabetic drugs and six treated with insulin), one with impaired glucose tolerance (IGT) while 12 were apparently normoglycemic (NGT).
The participants underwent a three-hour oral glucose tolerance test. P-glucose and p-insulin was measured at time = −15, −10, −5, 0, +15, 30, 45, 60, 90, 120, 180 minutes.
**Results:** The OGTT revealed that seven out of the 12 apparently NGT subjects had IGT, one had DM, only four having NGT.
Time for peak insulin response to glucose showed huge variance thus being evenly distributed from 30 to 180 minutes after the oral glucose load independent of glycemic status. In table 1, AUC, glucose and insulinogenic index is given according to the diabetic status of the participants.
**Conclusion:** Data show a vast heterogeneity in response to OGTT in patients with the mtDNA point mutation A3243G. In addition there was a large variance in the time of insulin peak values and insulinogenic indexes for the overall population.
Future studies will have to determine other factors which contribute to the observed huge variation in insulin secretion.
Seven out of 12 apparently NGT subjects had IGT and one had DM. Therefore it seems important to test the A3243G mutation positive subjects for DM regularly.
A negative correlation between DH in blood and the time of debut of DM or IGT was found. The degree of heteroplasmicity in blood may then serve as a prognostic factor for debut of DM.

Table 1: Insulinogenic index (IG-index): ΔAUC, insulin (0–180 min) / ΔAUC,glucose (0–180 min)

| | Insulinogenic index (pmol /L pr mmol /L) | AUC,glucose (0–180)(pmol /L × min) |
|---|---|---|
| Glycemic status | Median [Range] | Median [Range] |
| 9 DM | 5.1 [3.1–35.5] | 6362 [4637–7475] |
| 8 IGT | 24.5 [11.0–32.3] | 3252 [2175–4149] |
| 4 NGT | 19.4 [16.4–32.9] | 2096 [1972–2210] |

## 470

**Responses to intravenous glucose non-diabetic twins and healthy controls: a prospective follow-up study**

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**Background and aim:** To determine whether inherited changes in insulin secretion or sensitivity could predispose to type 1 diabetes we studied identical twins of patients with diabetes who are now unlikley to develop diabetes and compared them with a group of pre-diabetic twins.
**Research design and methods:** From our United Kingdom study of identical twins discordant for type 1 diabetes, we studied prospectively over a 20 year period, 27 non-diabetic twins and 16 controls. Of these twins 15 remain non-diabetic and at low disease risk, 12 went on to develop type 1 diabetes (pre-diabetic twins). All subjects were tested with an intravenous glucose tolerance test (IVGTT) on at least two occasions. Insulin secretion was measured as first phase insulin response (FPIR) to intravenous glucose. Insulin sensitivity was estimated by the homeostasis model of insulin resistance (HOMA-R) and by glucose clearance (Kg). Basal HOMA-R/FPIR ratio was calculated to evaluate insulin sensitivity according to insulin responses.
**Results:** Fasting blood glucose and insulin levels at the initial test were similar in low-risk non-diabetic twins mean ± SD (4.1 ± 0.2 mmol/l & 6.2 ± 3.7 mIU/ml respectively), and controls (4.6 ± 1.3 mmol/l & 3.6 ± 3.8 mIU/ml respectively) as well as on follow-up. In contrast, fasting insulin levels in the pre-diabetic twins were significantly higher than controls at the initial test (10.3 ± 5.9 vs3.6 ± 3.8 mIU/ml; $p<0.03$). Non-diabetic twins had similar FPIR as controls initially (500 ± 391 vs 450 ± 217 mIu/ml/10 min) and throughout the study while prediabetic twins had lower FPIR (245 ± 129 vs 450 ± 217 mIu/ml/10 min ; $p<0.03$). Glucose clearance (Kg) in the non-diabetic twins was similar to controls initially (mean 2.4 ± 1.0 vs 2.8 ± 1.0% per min) and on follow-up (mean 2.9 ± 1.4 vs 2.5 ± 0.8% per min); however Kg in pre-diabetic twins was lower compared to controls (1.6 ± 0.6 vs 2.9 ± 1.4% per min; $p<0.012$). While baseline HOMA-R/FPIR ratio did not differ between non-diabetic twins and controls, it was higher in pre-diabetic twins than controls (0.007 ± 0.005 vs 0.001 ± 0.0009 ; $p<0.02$).
**Conclusions:** These observations in non-diabetic twins failed to find evidence for a genetic or familial alteration in either insulin secretion or insulin sensitivity predisposing to type 1 diabetes. In contrast, pre-diabetic twins showed decreased insulin responses, glucose clearance and insulin sensitivity reflecting the acquired disease process.

# PS 26

# Deleterious effects of chronic high glucose on beta cells

## 471

**High glucose concentrations induce rat islet c-Myc and haeme-oxygenase 1 mRNA expression in an oxidative stress-dependent manner without activating the transcription factor NF-κB**

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**Background and aim:** Supraphysiological glucose concentrations and inflammatory cytokines involved in the development of type 1 diabetes, like interleukin 1β (IL1β), induce some alterations of similar nature on the β-cell phenotype. These include reduced expression of the β-cell specific insulin and PDX1 genes, increased expression of genes such as c-Myc and haeme-oxygenase 1 (HO1), and late apoptosis. These effects of IL1β partly result from receptor-mediated activation of NF-κB, and it has been suggested that the effects of hyperglycemia are also due to activation of this transcription factor by oxidative stress. In the present study, we tested the effects of high glucose and hydrogen peroxide on NF-κB activity in rat islets cultured in the presence or absence of the antioxidant N-acetyl-L-cystein (NAC).
**Materials and Methods:** Rat pancreatic islets were precultured for 1 week in RPMI medium containing 10 mmol/L glucose (G10) and 5 g/L bovine serum albumin instead of 10% fetal calf serum. They were then further cultured (for 30 min up to 8 days) in the presence of G10-G30 plus various test substances, and processed for measurement of islet NF-κB (p65) DNA binding activity (ELISA kit) or real-time RT-PCR determination of gene to TATA-box binding protein (TBP) mRNA ratios. Short-term exposure to IL1β (50 UI/mL) in the presence of G10 was used as a positive control condition for the activation of islet NF-κB.
**Results:** As expected, a 30 min exposure to IL1β consistently increased islet NF-κB DNA binding activity. Accordingly, islet mRNA expression of various NF-κB-dependent genes were markedly increased after 6 h exposure to the cytokine (~500, 30, 7 and 10 fold increase in iNOS, IκBα, c-Myc and HO1/TBP mRNA ratios respectively, n=3). In contrast, compared with culture in G10, exposure to G30 for 15–30 min, 1–8 h and 1–7 days did not increase islet NF-κB DNA binding activity, but induced an ~2–3 fold increase in insulin secretion. Although a sustained ~3–5 fold increase in c-myc and HO1/TBP mRNA ratios was observed after 1, 3 and 7 days of culture in G30, the islet iNOS and IκBα/TBP mRNA ratios remained unaffected under these conditions. Similar changes in islet gene mRNA expression and lack of effect on islet NF-κB DNA binding activity were observed after overnight exposure to 5 μM hydrogen peroxide in the presence of G10. Interestingly, the stimulation of islet HO1 mRNA expression by both G30 and hydrogen peroxide were abrogated by 1 mM NAC.
**Conclusions:** In contrast with IL1β, supraphysiological glucose concentrations and hydrogen peroxide do not activate rat islet NF-κB. The rapid and sustained stimulation of islet c-Myc and HO1 expression by G30 is ascribed to activation of a distinct, oxidative stress-dependent, signalling pathway.

## 472

**Distinct effects of the antioxidant N-acetyl-L-cystein on β-cell dysfunction induced by high glucose and hydrogen peroxyde in cultured rat islet**

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**Background and aims:** Chronic exposure to high glucose concentrations induces phenotypic alterations of pancreatic β-cells, including altered β-cell gene expression, reduced β-cell survival, and loss of glucose stimulation of insulin secretion (GSIS) within the physiological range of glucose concentrations. These alterations may result from an increase in oxidative stress under these conditions. To test this hypothesis, we compared the effects of high glucose and hydrogen peroxyde on key events of β-cell stimulus-secretion coupling in rat islets cultured in the presence or absence of the antioxidant N-acetyl-L-cystein (NAC).
**Materials and methods:** Rat pancreatic islets were cultured for 1 week in RPMI medium containing 10–30 mmol/L glucose (G10 or G30), 5 g/L bovine serum albumin instead of 10% fetal calf serum, and various test substances. They were then used for dynamic measurements of glucose-

induced changes in mitochondrial membrane potential (rhodamine 123 fluorescence), cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) (Fura-PE3 spectrofluorimetry) and insulin secretion, or processed for RT-PCR determination of gene to TATA-box binding protein (TBP) mRNA ratios.
**Results:** After one week culture in G30 instead of G10, the alterations of β-cell function were similar to those previously reported and included 1) an increased sensitivity to glucose for the acceleration of mitochondrial metabolism leading to maximal GSIS at low glucose concentration (6 rather than 20 mmol/L), 2) an ~50% reduction in islet insulin content, 3) a marked reduction in maximal GSIS, and 4) a lack of glucose-induced rise in $[Ca^{2+}]_i$. These alterations were accompanied by a ~3.5 fold increase ($p<0.001$) in islet mRNA expression of haeme-oxygenase 1 (HO1/TBP mRNA ratio), an antioxidant enzyme considered to be a sensitive indicator of oxidative stress in various cell types. In comparison with these effects of G30, overnight exposure of rat islets to 1–5 μM $H_2O_2$ in the presence of G10 also increased islet HO1 mRNA expression, but the functional alterations of β-cells were different : 1) a dose-dependent 2–3 fold increase in basal $[Ca^{2+}]_i$, 2) a decrease in rhodamine 123 loading in the periphery of the islets, and 3) the maintained ability of glucose to hyperpolarize the mitochondrial membrane in the center of the islets, and to increase $[Ca^{2+}]_i$ in the whole islets. When islets were exposed to G30 or $H_2O_2$ in the presence of 1 mmol/L NAC, the stimulation of HO1 mRNA expression was abrogated, suggesting that NAC effectively prevented islet oxidative stress under both culture conditions. However, whereas β-cell dysfunction induced by $H_2O_2$ was completely normalized by NAC, the alterations of β-cell function induced by G30 were not different from those in the absence of NAC.
**Conclusion:** The alterations of rat β-cell function after prolonged culture in high glucose concentrations do not seem to result from the increase in oxidative stress produced under these conditions.

## 473

**ACE-inhibition protects pancreatic human islets from glucotoxicity and improves the function of islets from Type 2 diabetic patients**
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**Background and aims:** The Renin-Angiotensin System (RAS) has been found locally in several tissues, including beta-cell lines. Since RAS activation is associated with an increase of reactive oxygen species (ROS) and in consideration of the fact that beta-cell damage induced by several factors (including high glucose = glucotoxicity) is mediated, at least in part, by ROS, we studied: 1) whether RAS is present in human islets of Langerhans (HI); 2) whether ACE-inhibition can reduce the damage induced in HI by glucotoxicity; 3) whether ACE-inhibition can affect the function of HI obtained from Type 2 diabetic (T2D) donors.
**Materials and methods:** HI were prepared from 10 non-diabetic, non-obese donors (ctrl; age: 49 ± 12 yrs, sex: 4M/6F, BMI: 24.9 ± 2.1 kg/m$^2$), and from 3 T2D donors (age 70.6 ± 5.1 yrs, sex: 2F/1M, BMI 26.7 ± 1.8 Kg/m$^2$). Messenger RNA expression of angiotensinogen, ACE and AT1 receptor in islet cells was demonstrated by quantitative RT-PCR.
**Results:** As expected, 24 h exposure of HI to high glucose (22.2 mM, G), determined a marked reduction of insulin secretion (stimulation index, SI: 2.7 ± 0.4 in ctrl and 1.1 ± 0.2 in G, $p<0.01$). This was associated with enhanced oxidative stress, as shown by the increase of specific markers (nytrotirosine: 7.0 ± 0.4 nM in ctrl and 14.8 ± 1.5 nM in G, $p<0.01$), and of the expression of PKC-beta2 (+40%) and NADPH-oxidase (+80%). The presence of an ACE-inhibitor (0.5, 1.0 or 3.0 mM Zofenoprilat) protected from glucotoxicity (SI: 1.7 ± 0.1, 1.9 ± 0.2 and 2.2 ± 0.6, all $p<0.05$ vs G; nytrotirosine: 9.3 ± 0.4, 8.7 ± 0.6 and 7.9 ± 0.25 nM, both $p<0.05$ vs G; decreased (–40 to –85%) PKC-beta2 and NADPH-oxidase expression). T2D islets also showed a defect of insulin secretion (SI: 1.0 ± 0.1, $p<0.05$ vs Ctrl), and an increase of oxidative stress (nytrotirosine: 10.1 ± 1.0 nM, $p<0.05$ vs Ctrl; PKC-beta2 and NADPH-oxidase expression: +25% and +50%, respectively). 24 h exposure of T2D islets to 0.5, 1.0 or 3.0 mM Zofenoprilat induced a significant (all $p<0.05$) improvement of insulin secretion (SI: 1.6 ± 0.2, 2.1 ± 0.3 and 2.1 ± 0.1), a reduction (all $p<0.05$) of nytrotirosine concentration (8.0 ± 0.3, 7.9 ± 0.02 and 7.5 ± 0.1 nM), and a decrease of PKC-beta2 (–35% to –42%) and NADPH-oxidase (–38% to –60%) expression.
**Conclusion:** These results show that: 1) RAS is present in HI; 2) ACE-inhibition protects HI from glucotoxicity; 3) ACE-inhibition improves the function of HI obtained from Type 2 diabetic patients; 4) the beneficial effects of ACE-inhibition are associated to a decrease of oxidative stress.

## 474

**Pioglitazone and sodium salicylate protect human beta-cells against apoptosis and impaired function induced by glucose and IL-1β**
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**Background and aims:** A progressive decrease in functional beta-cell mass characterises both type 1 and 2 diabetes. Among the underlying causes, beta-cell apoptosis and impaired secretory function seem to be partly mediated by immune- and/or high glucose-induced production of IL-1β in turn acting through the NFkB/Fas pathway. The aim of the present study was to determine whether two drugs that can block this pathway in some cell types, the thiazolidinedione pioglitazone and sodium salicylate, can protect human beta-cells against the toxic effects of IL-1β and high glucose in vitro.
**Materials and Methods:** Human islets were maintained in culture for up to 4 days at 5.5 mmol/l glucose with or without (control) 2–5 ng/ml IL-1β or at 33.3 mmol/l glucose. Glucose-stimulated short-term (1 h) insulin secretion was measured by radioimmunoassay at the end of the culture period. Cell death (indicative of apoptosis in this system) was assessed by TUNEL-positivity of beta-cells identified by immunocytochemistry. IL-1β secretion and NFkB activation were measured by ELISA. Data are mean±SE (n=3 independent experiments using separate donor pancreas) with statistical significance evaluated by Student's t-test.
**Results:** Culture for 4 days with IL-1β or 33.3 mmol/l glucose increased beta-cell apoptosis 3.1 ± 0.4 and 3.3 ± 0.7 fold respectively ($p<0.01$ vs. 5.5 mmol/l glucose: absolute value 0.38 ± 0.05 TUNEL-positive beta-cells/islet). After 4-day culture at 5.5 mmol/l glucose, subsequent short-term (1 hour) insulin secretion was stimulated 3.2 ± 0.4-fold by 16.7 mmol/l vs. 3.3 mmol/l glucose (absolute value at 3.3 mmol/l glucose = 0.75 ± 0.15 pmol/islet/hour) but 4-day culture with IL-1β or at 33.3 mmol/l glucose abolished subsequent glucose-stimulation of short-term insulin secretion. Neither pioglitazone (1–5 μmol/l) nor sodium salicylate (0.04 mg/ml) altered beta-cell apoptosis or short-term insulin secretion during culture at 5.5 mmol/l glucose. Both drugs prevented the increased apoptosis caused by culture with IL-1β or 33.3 mmol/l glucose and protected partially against the loss of glucose-stimulated insulin secretion. As expected, IL-1β secretion from islets was increased 5.0 ± 0.7-fold by 4-day culture at 33.3 mmol/l ($p<0.01$ vs. low 5 mmol/l glucose: absolute value 2.5 ± 1.0 pg/20 islets/4 days) and this was blocked by pioglitazone ($p<0.01$). NFkB activity was increased 1.6 ± 0.1-fold by 33.3 mmol/l glucose ($p<0.01$ vs. 5.5 mmol/l glucose) and both drugs prevented this increase.
**Conclusions:** Pioglitazone and sodium salicylate both protect human beta-cells against the detrimental impact on viability and insulin secretion of IL-1β and glucose (acting through IL-1β and NFkB) and may thus be useful in preventing or retarding decreased beta-cell mass in diabetes.
*Supported by: the Juvenile Diabetes Research Foundation.*

## 475

**The Fas pathway is involved in beta cell secretory function and is a target of glucotoxicity**
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**Background and aims:** Chronic hyperglycaemia is suggested to be detrimental to pancreatic beta-cells, causing impaired insulin secretion, apoptosis and decreased proliferation. We have shown that IL-1beta mediates part of these deleterious effects via an up-regulation of the Fas receptor. This led us to hypothesise that the Fas pathway may not solely induce changes in cell-turnover, but may also affect beta-cell secretory function.
**Materials and methods:** We tested our hypothesis in 6–7 week old Fas deficient mice (B6.MRL/Tnfrsf6$^{lpr}$) and beta-cell specific Fas knockout non-obese diabetic (NOD) mice (prior to onset of insulitis) with their respective controls. Beta cell function was assessed by intraperitoneal glucose tolerance test (IPGTT), *in situ* pancreatic perfusions and *in vitro* by an acute glucose stimulated insulin release assay. Insulin and Pdx expression were quantified by rtPCR. The functional role of Fas, by FLIP transfection. Beta cell mass was assessed by morphometry of tissue sections.
**Results:** Both Fas deficient and beta-cell specific Fas knockout mice had an impaired glucose tolerance. Circulating insulin levels were comparable between Fas deficient and wildtype mice, ruling out significant insulin

resistance. Pancreatic perfusions revealed that glucose-induced insulin secretion in Fas deficient mice was delayed (2 min), blunted (36%± 18% of controls, n=3), and abnormal (only 1 distinct phase). The islet surface area of Fas deficient mice was twice the size of wildtype islets ($p<0.05$), whereas insulin mRNA was decreased by 90% ($p<0.05$). The stimulation index of glucose-induced insulin secretion was reduced by 1.9-fold in islets isolated from Fas deficient mice compared to wildtype islets. Chronic exposure of wildtype islets to 33.3 mM glucose, decreased Pdx mRNA by 50%, insulin mRNA by 50% and acute glucose-stimulated insulin release by 3.7-fold ($p<0.05$) as compared to islets treated with 11.1 mM glucose. Up-regulation by transfection, of the caspase-8 inhibitor, FLIP, a mediator of Fas activation, prevented these deleterious effects without affecting beta-cell apoptosis or proliferation. Moreover, exposure of Fas deficient islets to elevated glucose concentrations did not significantly affect Pdx mRNA, insulin mRNA nor acute glucose stimulated insulin release. This could be attributed to the fact that the Fas deficient islets already have a similar phenotype to islets affected by glucotoxicity.
**Conclusion:** These results support a new role for the Fas pathway in regulating insulin production and secretion independently of changes in cell turnover and demonstrates its role in glucotoxicity.

## 476

**Mitochondrial proteins with potential role in INS-1E "glucotoxicity"**
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**Background and aims:** Hyperglycemia and impaired glucose-stimulated insulin secretion (GSIS) are hallmarks of type 2 diabetes mellitus (T2DM). Despite the link between the two parameters it is far from clear how elevated glucose concentrations contribute to secretory malfunction, i.e. the mechanisms of "glucotoxicity". Perturbations of different specific proteins of the β-cell involved in glucose metabolism have been demonstrated and coupled to impaired GSIS. In this context several mitochondrial proteins have been investigated. However, T2DM is multifactorial involving altered expression of numerous genes. In order to identify molecular mechanisms of "glucotoxicity" approaches allowing simultaneous measurements of multiple proteins are required. The aim was to investigate how elevated glucose concentrations, which cause impaired INS-1E secretory function, change the expression of multiple INS-1E proteins with special reference to the mitochondrial proteins.
**Materials and methods:** INS-1E cells (passage 90) were cultured in 24-well plates and in flasks for 5 days in RPMI 1640 containing 5.5, 11, 20 or 27 mM glucose. After the culture period cells in the 24-well plates were exposed to 3 or 11 mM glucose for 30 min. Insulin released to the media was measured by ELISA. Cells in the flasks were harvested and mitochondria isolated by subcellular fractionation. Mitochondrial proteins were extracted and applied on anionic (SAX2) and hydrophobic (H50) ProteinChip arrays. Protein profiling of the mitochondrial proteins was performed with surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS). Analysis was carried out by Ciphergen software.
**Results:** Insulin release from INS-1E cells, which had been cultured at 5.5 or 11 mM glucose, increased more than 3-fold compared to basal when acutely exposed to 11 mM glucose. In contrast, no change in insulin release was observed from INS-1E cells cultured at 20 or 27 mM glucose, when the glucose concentration was acutely elevated from 3 to 11 mM glucose. Mass spectra of lysates of mitochondria isolated from INS-1E cells cultured at 5.5, 11, 20 or 27 mM glucose contained more than 200 protein peaks in the 3 to 80 kDa mass range. When mass spectra obtained from mitochondria of INS-1E cells cultured at 11 mM glucose were compared with mass spectra obtained from mitochondria of INS-1E cells cultured at 27 mM glucose, 60 and 30 protein peaks were differentially ($p<0.05$) expressed with the anionic and hydrophobic arrays, respectively.
**Conclusion:** The SELDI-TOF MS proteomic approach is a powerful tool to discover multiple differentially expressed proteins in INS-1E cells, which were used as a model for the insulin-producing β-cell, by comparing cells cultured at normal and elevated glucose concentrations. It is anticipated that such proteins, when identified, will give new insights as to the molecular mechanisms of "glucotoxicity".
*INS-1E cells were kindly supplied by P Maechler and C Wollheim. The study was supported by an EFSD/JDRF/Novo Nordisk Type 2 Research Grant.*

## 477

**Islet proteins with potential role in hyperglycemia-induced initial enhanced insulin secretion and later insulin secretory failure of Type 2 diabetes mellitus**
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**Background and aims:** The pathophysiology of type 2 diabetes mellitus (T2DM) involves development of insulin resistance followed by insulin secretory failure. Whereas the former stage is associated with compensatory increased levels of insulin secretion, the latter stage is characterized by low secretory levels. In an attempt to address these multifactorial sequential events we have cultured islets at normal and elevated glucose concentrations for different time periods. The islet glucose-stimulated insulin secretion (GSIS) has been analyzed as well as the complex protein patterns of islets cultured under the different conditions.
**Materials and methods:** Islets were isolated from 5 months old male C57BL/6J-mice and cultured for 2 and 5 days in RPMI 1640 containing 5.5 or 20 mM glucose. After the culture period insulin secretion was measured by ELISA from islets perifused in the presence of 3 and 11 mM glucose. Also, proteins were extracted from islets cultured under the different conditions. The extracts were applied on anionic exchange surfaces. Protein profiling of the islet proteins was performed with surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS). Mass spectral analysis was carried out by supplied software.
**Results:** Whereas GSIS of normal magnitude was observed from islets cultured in the presence of 5.5 mM glucose for 2 days, elevated GSIS was observed from islets cultured at 20 mM glucose for 2 days. Mass spectral analysis of such islets revealed several peaks out which proteins with masses 22.8, 32.4 and 34.4 kDa were differentially ($p<0.05$) expressed when islets cultured at 5.5 mM glucose were compared with islets cultured at 20 mM glucose for 2 days. After 5 days culture GSIS from islets cultured at 5.5 mM glucose was similar to what was observed after 2 days culture. In contrast, culture in the presence of 20 mM glucose for 5 days reduced GSIS from islets to such an extent that secretory rates were lower than observed in islets cultured in the presence of 5.5 mM glucose. Mass spectral analysis of islets cultured for 5 days also revealed several protein peaks. A significantly higher expression of a 31.3 kDa protein was observed in islets cultured at 20 mM glucose compared to islets cultured at 5.5 mM glucose. The 22.8, 32.4 and 34.4 kDa proteins were equally expressed in islets cultured for 5 days.
**Conclusion:** Enhanced GSIS from C57BL/6J-mice islets, which is observed after culture at high glucose concentration, was associated with changes in several specific islet proteins determined by SELDI-TOF MS. After extended culture of islets at elevated glucose concentration, when GSIS decreases, other differentially expressed proteins appear. It is anticipated that identification of the former and latter sets of differentially expressed proteins will give new insights as to the molecular mechanisms of compensatory insulin hypersecretion and ensuing insulin secretory failure observed during the development of T2DM.
*Supported by: EFSD/Type 2 Diabetes Research Grant.*

# PS 27

# Lipotoxicity and mechanisms of beta cell damage

## 478

**Free fatty acids and cytokines induce pancreatic beta cell apoptosis by different mechanisms: role of NF-κB and endoplasmic reticulum stress**

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**Background and aims:** Increasing evidence suggests that a decrease in beta cell mass is a common feature of type 1 (T1DM) and type 2 (T2DM) diabetes mellitus. Apoptosis is probably the main form of beta cell death in both T1DM and T2DM. In T2DM, elevated levels of free fatty acids (FFA) released from the adipose tissue might contribute to beta cell dysfunction and apoptosis. In T1DM, cytokines (IL-1β, IFN-γ and TNF-α) probably contribute to beta cell destruction. We have previously shown that beta cell exposure to cytokines activates the transcription factor NF-κB, thereby inducing iNOS and chemokines such as MCP-1. The resulting NO formation triggers an endoplasmic reticulum (ER) stress. Other groups have suggested that high glucose and FFA exert their toxic effects on beta cells via NF-κB activation. Against this background, the aims of this study were: 1) to clarify whether common mechanisms are involved in FFA- and cytokine-induced beta cell apoptosis; 2) to determine whether TNF-α, an adipocyte-derived cytokine, potentiates the toxic effects of FFA through enhanced NF-κB activation.
**Materials and methods:** INS-1E cells and rat islets were cultured for 6–72 h in the presence or absence of FFA (oleate 0.5 mM or palmitate 0.25–0.5 mM, medium containing 1% BSA) and/or cytokines (IL-1β 30 U/ml, IFN-γ 100 U/ml, TNF-α 1000 U/ml). Cell death was examined by fluorescence microscopy using propidium iodide and Hoechst 33342 staining. Gene expression was analysed by real time PCR (corrected for GAPDH expression) and NF-κB activation by gel shift assay. The activation of XBP-1 via its alternative splicing (an indication of ER stress) was evaluated by restriction analysis following PCR amplification.
**Results:** Palmitate and IL-1β induced a similar percentage of apoptosis in INS-1E cells after 24–48 h (20–25% vs control 4–5%, n=5, $p<0.01$), while oleate was less toxic (8–11%, $p<0.05$). Palmitate also induced cell death in whole islets (40%) after 72 h, but to a lesser extent than IL-1β (70%). TNF-α did not potentiate FFA toxicity in INS-1E cells after 48 h (22% apoptosis for palmitate + TNF-α vs 25% for palmitate; n=4). The NF-κB-dependent genes iNOS and MCP-1 were not induced following exposure to FFA, whereas a clear induction was observed with cytokines (iNOS: $3.4 \pm 0.4$ after IL-1β vs $0.1 \pm 0.04$ in control; MCP-1: $0.9 \pm 0.25$ after IL-1β vs $0.01 \pm 0.01$ in control; n=5, $p<0.05$). Cytokines activated NF-κB in INS-1E cells, but FFA did not (n=3). Moreover, FFA did not enhance NF-κB activation by TNF-α (n=3). To assess whether FFA induce ER stress, the expression of CHOP and cleavage of XBP-1 were evaluated. After 6–48 h, palmitate and oleate induced CHOP mRNA expression (1.5–2-fold induction, n=5, $p<0.05$) and XBP-1 alternative splicing. Cytokines induced an earlier and more pronounced ER stress response.
**Conclusion:** Apoptosis is the main mode of FFA- and cytokine-induced beta cell death, but the mechanisms involved are different. While cytokines induce NF-κB activation, NO production and ER stress, FFA activate a milder ER stress response via an NF-κB- and NO-independent mechanism. Moreover, there were no synergistic effects between FFA and TNF-α. Our results argue against a unifying hypothesis for the mechanisms of beta cell death in T1DM and T2DM.
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**Triacylglycerol formed in islet beta cells exposed to fatty acids do not contribute to decreased viability except in cells forming predominantly tripalmitin**

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The causal factors for lack of glucose-stimulated insulin secretion (GSIS) following chronic exposure of beta cells to lipids are largely unknown; formation and accumulation of triacylglycerols (TG) or metabolic intermediates, induction of apoptosis and/or changes in stimulus-secretion coupling mechanisms are putative candidates.
**Aims:** To investigate the role of triacylglycerol formation from different non-esterified fatty acids (NEFA) in so-called beta cell "lipotoxicity" arising from chronic exposure of beta cells to lipids.
**Methods:** Clonal rat beta cells (INS-1) were exposed to NEFA (0.5 mM) at 3 and 20 mM glucose for 24 h. Following exposure to palmitic (16:0), oleic (18:1) and linoleic (18:2) acids or mixtures of NEFA, glucose stimulated insulin secretion (GSIS) was assessed. TG content and composition of TG and phospholipids were determined in cell extracts by gas chromatography. Cell viability was determined by quantification of propidium iodide positive nuclei. Morphological changes were assessed by light (LM) and electron microscopy (EM).
**Results:** Incubation of INS-1 cells with NEFA (0.5 mM) resulted in increased basal insulin secretion (from 5ug/ml insulin in control to over 11 mg/ml insulin per mg of cell protein in NEFA treated cells, ANOVA $p=0.05$) but loss of GSIS. Intracellular TG concentration increased following exposure to all NEFA's. The degree of accumulation increased up to 4 fold at 20 mM glucose compared to that at 3 mM glucose. TG at 20 mM glucose was highest with oleic acid ($6.0 ng/10^5$ cells) and lowest with palmitic acid ($2.4 ng/10^5$cells) (ANOVA; $p=0.05$) although more palmitic acid formed TG when cells were exposed to palmitic/oleic mixtures ($3.8 ng/10^5$cells). The phospholipid composition (PL) was altered following incubation in 0.5 mM palmitic acid, an effect increased by glucose (16:0 to 18:2 ratio, 3.08–6.21 mg PL fatty acids/mg protein). Cytotoxicity was not present at 0.5 mM NEFA but was increased at higher concentrations of palmitate (1 mM) and glucose (20 mM). Lipid droplets were visible in the cells by LM and EM following incubation with linoleic and oleic acids. Palmitic acid alone induced angular lacunae in the cytoplasm but no visible droplets.
**Conclusions:** Decreased glucose sensitivity of beta cells following chronic exposure to lipids ("lipotoxicity") occurs independently of degree of saturation of NEFA and is not associated with cell death. Lack of GSIS in hyperglycaemia and hyperlipidaemia could be mediated via accumulation of TG or intermediates of lipid metabolism. Palmitic acid causes deleterious morphological changes in beta cells, which are reduced by addition of oleic/palmitic mixtures. These toxic effects are likely to be the result of in vitro intracellular formation of structurally rigid tripalmitin (melting temp 65°C) and phospholipid membranes rather than a biologically relevant metabolic effect of palmitic acid.
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**Adiponectin does not prevent lipoapoptosis of human islets**

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**Background and aims:** Fatty acid-induced apoptosis (lipoapoptosis) of beta-cells plays an important role in the development of type 2 diabetes. Recently, it has been shown that the adipocytokine adiponectin exerts anti-lipoapoptotic effects in rat insulinoma cells. Therefore, we studied whether human islets express receptors for adiponectin (AdipoR1 und AdipoR2) and whether adiponectin is able to exert anti-lipoapototic effects in human beta-cells.
**Materials and methods:** Human islets were isolated from pancreata of three different multiorgan donors. mRNA expression of AdipoR1 und AdipoR2 was quantified by real-time RT-PCR. Apoptosis was assessed by flow cytometric cell cycle analysis (subG1-formation).
**Results:** Human islets expressed 2.3-fold more AdipoR2 than AdipoR1 mRNA (means: 852 vs 369 fg/μg total RNA). Treatment of human islets with stearate (1 mM, 72 hrs) induced a 3.3-fold increase in apoptosis compared to control cells. Incubation of human islets with palmitate (1 mM, 72 hrs) also triggered apoptosis significantly but to a much lower extent than stearate (1.7-fold increase over basal). Treatment of human islets with adiponectin (5 μg/ml, 72 h) had no significant effect on basal apoptosis or lipoapoptosis.
**Conclusion:** Taken together, human islets express both adiponectin receptors. However, adiponectin neither influences basal apoptosis nor exerts anti-lipoapoptotic effects. Therefore, the role of adiponectin in human islets remains unclear and awaits further studies.

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**Pioglitazone protects human islets from lipotoxicity improving intracellular lipid metabolism and activating phosphoinositol-3-kinase**

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**Background and aims:** We have recently shown that PPAR-gamma2 agonists (Prostaglandin-J2 and rosiglitazone) protect cultured human islets from the cytostatic effects of elevated concentrations of free fatty acids (FFA). In order to explore the mechanisms contributing to such protective effect, we evaluated lipid metabolism of pancreatic islet as well as activation of phosphoinositol-3-kinase (PI3K) upon exposure of human islets to high FFA levels with and without pioglitazone.
**Materials and methods:** Human islets were isolated from 7 non-obese multiorgan donors (age 67.3 ± 4.7 yrs, sex 4M/3F, BMI 24.6 ± 1.8 Kg/m$^2$) by collagenase digestion and density gradient purification. All experiments were carried out 3–5 days from isolation.
**Results:** 24 h exposure to 1.0 mM FFA (oleate:palmitate, 2:1; 6% human albumin) determined a marked increase of apoptosis (O.D., arbitrary units: 0.94 ± 0.04 vs. 0.32 ± 0.02, $p<0.01$), a reduction of the ratio of stimulated over basal insulin release (Stimulation Index, SI: 1.0 ± 0.1 vs. 2.5 ± 024, $p<0.01$), total insulin content (776.8 ± 26 vs. 1852 ± 112 μU/ml, $p<0.05$), and reduction in insulin mRNA expression (–70%) as well as mRNA expression for PPAR-gamma2 (–74%), PI3K (–69%), PDX-1 (–70%), and LPL (–30%), all determined by quantitative Real-Time RT-PCR. FFA inhibited lipid oxidation (–40%) and increased islet triglycerides content (+100%; both $p<0.05$). Moreover, FFA decreased significantly the IRS1 (–32%) and IRS2 (–45%) activated protein expression measured by immunoblotting studies ($p<0.05$). FFA effects were completely prevented by concomitant islet incubation with 6.0 μg/ml pioglitazone, as indicated by normalization of insulin secretion, total insulin content (+312% and +318%, respectively), amount of apoptosis (-56%), lipid oxidation (+76%), and triglycerides content (–66.5%). Moreover, pioglitazone significantly increased insulin (+190%), PI3K (+200%), PDX-1 (+220%), LPL (+310%), and PPAR-gamma2 (+110%) mRNA expressions. Finally, pioglitazone normalized the IRS1 (+55%) and IRS2 (+47%) activated protein expression (all $p<0.05$ or less).
**Conclusion:** In conclusion, these results suggest that pioglitazone exerts a protective effect in islets exposed to FFA, mediated by the normalization of intracellular lipid accumulation, and through activation of PI3K-mediated insulin signaling pathway.

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**The antioxidant taurine prevents lipotoxicity on beta cell function *in vitro* and *in vivo***

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**Background and aims:** Prolonged elevation of plasma free fatty acids (FFA) has an impairing effect on both β cell function and turnover (β cell lipotoxicity), which may play an important role in the pathogenesis of type 2 diabetes. The mechanisms of this effect are still unclear; however, oxidative stress has been shown to play an important role in glucotoxicity, i.e. the impairment in β cell function and turnover induced by glucose, which is in many aspects similar to that induced by fatty acids. Our objective was to investigate the potential role of oxidative stress in β cell lipotoxicity.
**Materials and methods:** To determine whether antioxidants prevent the impairing effect of prolonged elevation of plasma FFA on β cell function, we coinfused i.v. oleate (1.3 μmol/min), for 48 hours, with the antioxidants Taurine (TAU, 0.35 mg kg$^{-1}$ min$^{-1}$) or N-acetyl-cysteine (NAC, 0.35 mg kg$^{-1}$ min$^{-1}$) in Wistar rats. Six groups of rats were studied: saline-treated rats as controls (SAL), oleate-treated rats (OLE), rats treated with oleate + taurine (OLE + TAU) or oleate + N-acetyl-cysteine (OLE + NAC), and rats treated with N-acetyl-cysteine alone (NAC) or Taurine alone (TAU). Islets were isolated to evaluate insulin secretion *in vitro*, or hyperglycemic clamps were performed to evaluate glucose stimulated insulin secretion *in vivo*.
**Results:** Glucose-stimulated insulin secretion in isolated islets was less with oleate ($p < 0.05$) but was restored by the addition of taurine at both 13 mM (p = NS, SAL vs. OLE + TAU or TAU) and 22 mM (SAL = 1.5 ± 0.2 pmol per islet per h, OLE = 1.0 ± 0.1, $p < 0.01$ vs. SAL; OLE + TAU = 1.7 ± 0.2, TAU = 1.9 ± 0.2, both p = NS vs. SAL), whereas NAC had only a partial effect (OLE + NAC = 1.2 ± 0.2, p = NS vs. SAL or OLE; NAC = 1.6 ± 0.2). Similarly, the insulin and C-peptide responses to the hyperglycemic clamp, which were reduced by oleate ($p < 0.05$), were restored by the addition of taurine at both 13 mM (p = NS, SAL vs. OLE + TAU or TAU) and 22 mM (C-peptide: SAL = 6.3 ± 0.5 nM, OLE = 3.8 ± 0.5, $p < 0.01$ vs. SAL; OLE + TAU = 5.6 ± 0.8, TAU = 6.3 ± 0.4, both p = NS vs. SAL), whereas NAC + OLE prevented 57.5% of the decrease in the C-peptide response observed with OLE alone.
**Conclusion:** We conclude that taurine prevents oleate induced lipotoxicity on β cell function, by a mechanism that may be linked to its antioxidant effect.
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**Protein expression in pancreatic islets exposed to fatty acids with different chain length and degree of saturation**

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**Background and aims:** Long-term exposure of pancreatic islets to free fatty acids (FFA:s) reduces glucose-stimulated insulin secretion (GSIS). However, the potency of different fatty acids to diminish GSIS is influenced by their degree of saturation. Whereas saturated FFA:s effectively counteracts GSIS, unsaturated FFA:s have been reported to have a protective effect on GSIS. The mechanisms for these findings are to a large extent unknown. By using a proteomics approach that combines protein-surface interactions and time-of-flight mass spectrometry (TOF MS) we have previously, in pancreatic islet cell lysates, observed proteins whose expression levels are regulated by oleic acid. The masses of these proteins are 7.0 kDa, 10.2 kDa, 12.2 kDa, 12.5 kDa, 25.0 kDa, 26.0 kDa and 28.5 kDa. The aim of the present study was to analyze the expression levels of these proteins in islets cultured at 11 mM glucose with or without different fatty acids. The fatty acids were chosen to vary both with regard to chain length and degree of saturation.
**Materials and methods:** Islets from male C57black/6J mice were isolated and cultured for 48 hours in RPMI 1640 culture medium prepared with or without 0.4 mM of either caprylic acid (C8:0), lauric acid (C12:0), palmitic acid (C16:0), oleic acid (C18:1) or linoleic acid (C18:2) in complex with 0.5 mM BSA. After culture islets were tested for their GSIS capacity in response to11 mM glucose under normal or depolarized (400 μM diazoxide and 30 mM KCl) conditions. Also, proteins were extracted from islets cultured under the different conditions. The extracts were diluted in 0.1 M phosphate buffer pH 6 supplemented with 0.1% Triton X-100 and applied on anionic exchange surfaces. Proteins were then analyzed with TOF MS.
**Results:** All fatty acids except C8:0 reduced GSIS under normal conditions by at least 50% ($p<0.05$). Under depolarized conditions only islets cultured with C16:0 or C18:1 displayed a significant reduction (60%, $p<0.05$) in secretory capacity. The correlations between GSIS and the expression levels of the previously discovered proteins were calculated. For the 25.0 and 28.5 kDa proteins the expression levels correlated positively (25.0 kDa) and negatively (28.0 kDa) with GSIS under normal as well as under depolarizing conditions. For the 10.2 and 12.2 kDa proteins the expression levels were positively (10.2 kDa) and negatively (12.2 kDa) correlated with GSIS under depolarizing conditions. For the 7.0 kDa and the 26.0 kDa protein expression levels could not be correlated to GSIS under any condition. In this study we were unable to detect the 12.5 kDa protein.
**Conclusion:** The potency of different fatty acids to affect GSIS is shown to correlate with variations in expression levels of certain proteins. Increasing chain length for saturated fatty acids is associated with more inhibition of GSIS. This is particular evident for GSIS under depolarizing conditions. The fact that the expression levels of the 25.0 kDa and the 28.5 kDa protein correlated with GSIS under both normal and depolarizing conditions indicated that these proteins are involved in both the K-ATP-channel dependent and independent pathway. On the other hand the 10.2 kDa and the 12.2 kDa protein are likely to be involved only in the K-ATP-channel independent pathway.

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**Induction by long-term elevated glucose of UCP-2 mRNA in rat pancreatic islets is coupled to increased fatty acid oxidation but not to effects on insulin secretion**

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**Background and aims:** An increase in uncoupling protein-2 (UCP-2) in insulin-producing beta cells can attenuate insulin secretion. Up-regulation by hyperglycemia of UCP-2 could thus be a factor behind diabetes-induced beta cell dysfunction; however regulation and functional consequences of such an effect have not been clarified. We investigated regulation by

long-term elevated glucose in vitro on UCP-2 mRNA and parallel functional changes in rat pancreatic islets.
**Materials and methods:** Rat pancreatic islets were cultured for a 48-h period with 27 or vs. 5.5 mm glucose with various additions.
**Results:** Culture at 27 mM glucose enhanced UCP-2 mRNA by 40%, $p<0.05$ and concomitantly enhanced carnitine palmitoyl transferase 1 (CPT-1) mRNA by 31%, $p<0.05$. The glucose effect on UCP-2 was abrogated not only by the mitochondrial uncouplers DNP and FCCP but also by a specific inhibitor of CPT-1 activity, Etomoxir (10 mikroM). Etomoxir depressed C14- oleate oxidation by 48%. Co-culture with 325 mikroM diazoxide abrogated the glucose effects on UCP-2 as well as on CPT-1 mRNA. Co-culture with diazoxide did not affect C14 glucose oxidation but inhibited post culture C14 oleate oxidation by 47%. 5-hydroxydecanoate (5-HD), a recognized antagonist of diazoxide on mitochondrial function, nullified the abrogating effect of diazoxide on glucose-induced UCP-2 mRNA and concomitantly reversed the inhibitory effect by diazoxide on oleate oxidation. The antioxidants vitamin E, lipoic acid or N-acetylcysteine failed to influence the glucose effect on UCP-2. No parallelism was found between glucose-induced insulin secretion and conditions which affected the glucose-induced rise in UCP-2 mRNA.
**Conclusion:** 1) long term elevated glucose enhances UCP-2 expression by an effect coupled to increased fatty acid oxidation and, 2) the glucose effect on UCP-2 does not translate into effects on insulin secretion
*Supported by: Norwegian Research Council*

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**Adiponectin acutely modulates insulin secretion in mice with high-fat diet induced insulin resistance**
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**Background and aims:** Obesity with insulin resistance is a major risk factor for the development of type 2 diabetes. Adipose tissue is central for this risk because the tissue is enlarged and secretes several factors that have been found to be involved in maintaining glucose homeostasis. One such factor is adiponectin, the levels of which are decreased in several models of insulin resistance. Adiponectin receptors were recently identified on pancreatic β-cells, and adiponectin has been suggested to counteract cytokine- and fatty acid-induced apoptosis in pancreatic β-cells. However, whether adiponectin has a role in the regulation of insulin secretion is not known. The aim of this study was to examine whether adiponectin exerts any acute effects on islets isolated from normal or insulin resistant mice.
**Materials and methods:** Islets were isolated from female C57BL/6J mice that were fed either a normal, low-fat diet (11% by energy) or a high-fat diet (58% by energy), which induces insulin resistance. For insulin secretion studies, freshly isolated islets were incubated at 2.8 mM or 16.7 mM glucose with and without addition of 5 μg/ml adiponectin (full-length recombinant mouse adiponectin) for 1 hour. For fuel oxidation, islets were incubated with $^{14}$C-glucose or $^{14}$C-palmitate, in 2.8 mM or 16.7 mM glucose, with and without addition of 5 μg/ml adiponectin and the production of $^{14}CO_2$ was measured after 2 h incubation. The ATP/ADP ratio was measured in overnight cultured islets under similar conditions as above, i.e., after incubation in 2.8 mM or 16.7 mM glucose, with and without adiponectin.
**Results:** In islets from normal mice, adiponectin had no significant effect on insulin secretion. In contrast, in islets from insulin resistant mice, adiponectin inhibited insulin secretion at 2.8 mM (75 ± 11 pmol/l versus 164 ± 27 pmol/l, $P<0.01$) but augmented insulin secretion in 16.7 mM glucose (1900 ± 380 pmol/l versus 1040 ± 120 pmol/l, $P<0.05$). This augmentation of glucose-stimulated insulin secretion (GSIS) by adiponectin was accompanied by increased glucose oxidation (4.2 ± 0.3 pmol/islet/h versus 2.8 ± 0.2 pmol/islet/h, $P<0.005$), and slightly reduced palmitate oxidation but unaltered ATP/ADP ratio. In normal islets, adiponectin did not affect glucose oxidation or ATP/ADP ratio but palmitate oxidation was slightly reduced.
**Conclusion:** Adiponectin has a glucose-dependent dual effect on insulin secretion in islets from insulin-resistant (high-fat fed) mice in that the adipokine decreases basal insulin secretion but potentiates GSIS. This effect may be secondary to fuel partitioning with reduced fatty acid but increased glucose oxidation. In contrast, in normal islets adiponectin does not appear to have any acute effects on insulin secretion. The results thus uncover a potential role for adiponectin to modify insulin secretion in insulin resistance, which supports a role of adiponectin as a potential signal involved in islet compensation to insulin resistance.

# PS 28

# In vitro studies on beta cell damage, degeneration and apoptosis

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**TNFalpha impairs insulin signaling in pancreatic beta-cells through JNK-dependent serine phosphorylation of IRS-2**
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**Background and aims:** TNFalpha represents an important mediator of beta-cell dysfunction and loss in autoimmune diabetes. In some cell lines, TNFalpha promotes IRS-1 phosphorylation on serine residues, leading to impairment of insulin and IGF-I signaling. Since IRS-1 and IRS-2 play an important role in beta-cell secretory function and survival, the purpose of this study was to evaluate the effects of TNFalpha on IRS signaling in pancreatic beta-cells.
**Materials and methods:** Two murine beta-cell lines, INS-1 and NIT-1, were stimulated with 20 ng/ml TNFalpha for various times, and phosphorylation of IRS proteins was evaluated by immunoprecipitation with specific antibodies followed by immunoblotting with phosphotyrosine or phosphoserine antibodies. The biological efficacy of TNFalpha in INS-1 and NIT-1 cells was confirmed by its capacity to transiently decrease the cellular levels of IkappaBalpha, which mediates activation of the transcription factor NF-kappaB.
**Results:** In both INS-1 and NIT-1 cells, TNFalpha increased the phosphoserine content of IRS proteins with a peak after 15 and 30 min of stimulation ($P<0.05$), but did not change the total protein levels of IRS-1 or IRS-2. Sequential immunoprecipitation with anti-IRS-1 or anti-IRS-2 antibodies, followed by immunoblotting with phosphoserine antibodies, demonstrated that IRS-2 was the most important substrate protein affected by TNFalpha, because serine phosphorylation of IRS-1 could be barely detected. Furthermore, TNFalpha treatment markedly inhibited IRS-2 tyrosine phosphorylation in response to insulin. In both beta-cell lines, TNFalpha stimulation resulted in increased activation of the serine-kinase JNK, and pre-incubation of cells with the JNK inhibitor SP600125 for 2 h prevented IRS-2 serine phosphorylation in response to TNFalpha. Finally, TNFalpha-mediated serine phosphorylation of IRS-2 and inhibition of insulin-stimulated tyrosine phosphorylation of IRS-2 were markedly attenuated when beta-cells were cultured in medium containing low (0.5 mM) as compared to high (11 mM) glucose concentrations.
**Conclusion:** TNFalpha increases serine phosphorylation of IRS proteins in pancreatic beta-cells, with greater effect on IRS-2 compared to IRS-1. The enhanced serine phosphorylation of IRS-2 inhibits its subsequent tyrosine phosphorylation by insulin. The TNFalpha effect is mediated by JNK and augmented by high glucose in the culture medium. These results suggest that TNFalpha may cause beta-cell dysfunction and death by interfering with insulin signaling at the level of IRS-2.

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**Secretagogin in beta cells – potential role and relevance for Type 1 diabetes mellitus**
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**Background and aim:** Secretagogin is a calcium-binding protein influencing calcium influx and cell proliferation, but the exact function is unknown. Secretagogin is expressed in neuroendocrine cells with highest expression in islet of Langerhans. It has high similarity to the widely expressed calcium-binding protein calbindin D-28 k, which when over-expressed, can inhibit cytokine induced β-cell apoptosis. Exposure of diabetes prone BB rat islets to IL-1β *in vitro* down-regulates secretagogin.
The aims of the study were to investigate: 1) the effect of over-expression of secretagogin in cytokine exposed RIN-5F cells and to 2) examine the effect of secretagogin on genes involved in β-cell function and cytokine mediated destruction.
**Materials and methods:** RIN-5F cells stably over-expressing secretagogin were exposed to cytokine mix (1.500 pg/ml IL-1β, 40 U/ml IFN-γ and 200 U/ml TNF-α). Released nitric oxide (NO) and insulin were measured after 72 hours of cytokine exposure as well as apoptosis by the Cell death detection ELISA plus assay. To investigate the effects of secretagogin

over-expression in RIN-5F cells on cytokine influenced gene expression, RIN-5F cells were exposed to cytokine mix for 24 hours, n=3. Cells were snap frozen and RNA were extracted with Trizol and the expression of 25 genes such as iNOS, IkB, Glut 2 and calmodulin were analyzed by semi-quantitative RT-PCR. Activation status for ERK1/2, p38 and JNK1/2 were analysed by Western blotting of RIN-5F cells exposed for cytokine mix for 1/2, 1, 3 and 6 hours, n=3.
**Results:** Transfected RIN-5F cells released significant lower NO (282 vs. 420 percentage of control) in response to cytokine mix compared to control cells (n=6, p=0.015). No differences were observed in insulin release or apoptosis. Analysis by RT-PCR revealed down-regulation of iNOS, IkB, PKB, CHOP/GADD and GLUT2 in transfected RIN-5F cells exposed to cytokine mix compared to cytokine exposed non-transfected cells. Calmodulin was up-regulated in cytokine exposed transfected RIN-5F cells compared to non-transfected cells. Cytokine-induced phosphorylation of ERK1/2 protein was reduced in transfected RIN-5F cells and the iNOS protein response was delayed in response to cytokine mix exposure. No differences were observed in the phosphorylation of JNK and p38.
**Conclusion:** Over-expression of secretagogin reduces both phosphorylation of ERK1/2, iNOS gene transcription, iNOS protein and NO production in RIN-5F cells in response to cytokine mix exposure. This suggests that changes in the secretagogin expression level influences the IL-1β NO dependent apoptosis pathways in RIN-5F cells and combined with its tissue specificity it could be a potential candidate gene for Type 1 diabetes.

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**Human beta cell specific enteroviral infection induces pro-apoptotic changes as evidenced by microarray gene expression profiling**
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**Background and aims:** Epidemiological evidence suggests that enteroviral infections may contribute to type 1 diabetes (T1DM) pathogenesis, although the effect(s) of enteroviral infection of the beta-cell remain to be elucidated. Our previous observation on pancreas from T1DM patients showed signs of beta-cell specific enteroviral infection with non-destructive insulitis and increased expression of proinflammatory molecules such as FAS, IFN alpha, IP10 and MCP1. To explore the hypothesis whether such infection can by itself affect beta-cell integrity, we studied the effects of enteroviral infection on islets obtained from non-diabetic organ donors, outside the immunopathological environment of the insulitic pancreas where these effects have so far been studied. To this end, an enterovirus was extracted from a T1DM pancreas and viral replication occurred in KB permissive cells. The virus was then used to infect human pancreatic islets isolated from non-diabetic pancreas. Occurrence of enteroviral infection was confirmed by electron microscopy that showed a beta-cell specific tropism of this in-vitro infection, in accordance to ex-vivo evidence. In addition, morphological features of cell suffering characterized by picnotic nuclei and signs of mithocondrial damages were present.
**Materials and methods:** Consequently, we analyzed, by macroarray technology, the gene expression profiling of in vitro infected islets compared with uninfected islets.
**Results:** The screening of more than 300 genes involved apoptosis regulation showed that enteroviral infection downregulates several molecules promoting cell division such as cyclins and cyclin-dependent kinases (CDK). Of note, such analysis uncovered an up-regulation of pro-apoptotic molecules such as FAS, GADD45, BAD, Caspase 9 and CD40L. On one side, the increased levels of Caspase 9 are in accordance with the morphological signs of motochondrial damage; while, on the other, the unchanged levels of Caspase 3, the key regulator of apoptosis execution well parallels the lack of morphological signs of apoptosis.
**Conclusions:** In conclusion our results demonstrate that human islet enteroviral infection is beta-cell specific and determines cell suffering characterized by mitochondrial damage and upregulation of proapoptotic genes that, in the presence of proinflammatory immune-mediated events, may lead to beta-cell destruction.

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**Endoplasmic reticulum stress may be involved in the beta cell death in calmodulin-overexpressing transgenic mice**
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**Background and aims:** Calmodulin is one of the most abundant intracellular proteins in the pancreatic beta cell. Its overexpression in the beta cell causes massive apoptotic cell death in vivo, resulting in obvious hyperglycemia. We previously suggested that $Ca^{2+}$/calmodulin-dependent NO production participates in the beta cell apoptosis. The beta cell is known to be susceptible to nitric oxide (NO)-induced endoplasmic reticulum (ER) stress, which may be involved in the pathogenesis of beta cell dysfunction in type 2 diabetes. The aim of this study was to examine whether beta cell death in the calmodulin transgenic mice was due to ER stress and a resultant cellular damage.
**Materials and methods:** Calmodulin was overexpressed in vivo in a beta-cell specific manner under the control of the rat insulin II promoter gene. Islet size was manually measured using hematoxilin/eosin-stained images of the pancreas. The alpha and beta cell masses were quantified using an image analyser by immunostaining with anti-insulin and anti-glucagon antibodies, respectively. Pancreatic islets were isolated by collagenase digestion, and the islet expression of chaperone proteins such as GRP78 and GRP94 and the ER stress protein CHOP was examined by RT-PCR. The contents of insulin in the whole pancreases and those of calmodulin in the isolated islets were determined by ELISA and RIA, respectively.
**Results:** Pancreatic islets from the transgenic mice were slightly bigger than those from non-transgenic littermates at one week of age. However, it only marginally increased in the following several weeks, whereas non-transgenic islets demonstrated a remarkable increase in size thereafter. These changes were consistent with the time-dependent changes in the beta cell mass and the pancreatic insulin contents. The alpha cell mass was also decreased in the transgenic islets to a lesser extent. The messenger RNA levels of GRP78, GRP94 and CHOP were siginificantly elevated in the transgenic islets. Calmodulin expression in the transgenic islets was 60% higher than that in the non-transgenic ones.
**Conclusion:** A small increase in the islet calmodulin expression levels may induce beta cell damage in the transgenic mice via an ER stress-dependent mechanism. The alpha cell mass may be influenced by the fate of the beta cell.

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**Identifying peptide-based inhibitors of human islet amyloid polypeptide (hIAPP) fibrillogenesis**
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**Background and aims:** Following the identification of human islet amyloid polypeptide (hIAPP) as a key component of pancreatic amyloid, research efforts have focused on determining the residues in hIAPP which are responsible for its amyloidogenic properties. It has been well established that the 20–29 domain of hIAPP is required for fibrillogenesis and cytotoxicity. More recent studies have identified several other domains that are also capable of accelerating fibrillogenesis by full-length hIAPP. We have now identified two new peptide fragments that are able to interact with full-length hIAPP, inhibit fibrillogenesis, and reduce IAPP-mediated cytotoxicity in culture.
**Materials and methods:** Circular dichroism spectroscopy was used to measure the peptide conformational changes associated with fibril formation. Negative stain electron microscopy was used to visualize the relative density and morphology of fibrillar structures that were obtained. IAPP toxicity assays were carried out by incubating RIN1056A cells in the presence of IAPP alone, or with inhibitory peptides.
**Results:** Circular dichroism spectroscopy revealed two hexapeptide fragments derived from IAPP that were strong inhibitors of beta-sheet formation and amyloid aggregation. These peptides were capable of preventing the conversion of hIAPP from a random coil to a beta-sheet conformation. Electron microscopic analyses revealed that co-incubation of these peptides with IAPP resulted in the loss of the typical high density and morphology of the amyloid fibrils. However, differences in activity were noted as only one of peptides was able to prevent IAPP-mediated cell death.
**Conclusion:** The present studies were designed to outline further the importance of specific domains within IAPP that are involved in its fibrillogenic and cytotoxic properties. The results presented here suggest that smaller fragments derived from these domains can interact with full-length

hIAPP to inhibit fibrillogenesis and cytotoxicity. These data emphasize the role of multiple domains when discussing the amyloidogenic properties of the human IAPP molecule as a whole. The purpose of these studies is to identify potential peptide inhibitors of hIAPP fibril formation to be used as a part of an overall therapeutic approach to combat complications due to the loss of functional beta-cell mass in type 2 diabetes.
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**Examining the role of gelsolin in insulin secretion and cell survival in the mouse pancreatic beta cell line MIN6**
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**Background and aims:** Gelsolin is a $Ca^{2+}$-dependent actin severing protein implicated in different biological activities such as dynamic rearrangements of the cytoskeletal architecture and apoptosis. Given that the actin cytoskeleton has been proposed to play a major role in insulin secretion and β cell survival, we decided to investigate the role of gelsolin in these two crucial aspects of β cell function in the mouse pancreatic insulin-secreting MIN6 cell line.
**Materials and methods:** The experiments were performed in two different sublines of the MIN6 cell line, namely B1 and C3, previously subcloned by our group and shown to differ in their secretory properties. The level of expression of gelsolin in these two sublines was analysed by microarray, real time RT-PCR and Western blot. Apoptosis in B1 vs. C3 cells was measured with a cell death detection ELISA kit after 48 hours culture in media containing different glucose and serum concentrations. The plasmid pSUPER-GFP-gelsolin RNAi-1, encoding an shRNA specific for mouse gelsolin, was successfully used to knock-down this protein by RNA interference in the B1 subline. Secretion was analysed 3 days after co-transfecting B1 cells with pSUPER-GFP-gelsolin RNAi-1 and a human growth hormone-expressing vector. The amount of secreted human growth hormone was subsequently measured using a human growth hormone ELISA kit. The levels of apoptosis in gelsolin-depleted vs. control B1 cells were analysed by ELISA following GFP-positive FACS. Data are means ± SEM.
**Results:** Gelsolin was found to be over-expressed in the B1 compared to the C3 subline by microarray analysis (145.7 fold increase ± 48.9, n=3), real time RT-PCR (115.8 fold increase ± 31.7, n=3) and Western blot. In addition, higher levels of apoptosis were detected in the C3 compared to the B1 subline under normal culture conditions (4.3 ± 1.7-fold C3 vs. B1, n=4, $P<0.04$). This difference was accentuated under low glucose and serum pro-apoptotic culture conditions (5.7 ± 1-fold C3 vs. B1, n=4, $P<0.01$). Depletion of gelsolin by RNA interference in the B1 subline was confirmed by Western blot and immunofluorescence. Human growth hormone secretion measurements showed a 36% reduction in the secretory response to 16.7 mM glucose in gelsolin-depleted vs. control B1 cells (Gelsolin-depleted cells: 2.9 fold stimulation ± 0.3; Control cells: 4.6 fold stimulation ± 0.6; n=6, $P<0.04$ from 2 independent experiments). Finally, preliminary results show that there is an increase in the level of apoptosis in gelsolin-depleted B1 cells as compared to the controls.
**Conclusion:** Our results point towards a role for gelsolin in the maintenance of regulated secretion, and an anti-apoptotic function for this protein in the MIN6 cell line.
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**Expression of protein kinase G and cGMP-phosphodiesterase isoforms in pancreatic β-cells and investigation of the roles of these enzymes in control of cell viability**
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**Background and aims:** Recent studies have suggested that the cGMP/protein kinase G signalling pathway may be involved in the regulation of viability in pancreatic β-cells. In support of this, it has been shown that the NO-independent activator of soluble guanylyl cyclase, YC-1, caused a time- and dose-dependent increase in β -cell death and that this correlated with cGMP production. This response was attenuated by inhibitors of protein kinase G (PKG) suggesting that PKG may be an important regulator of β-cell viability. However, PKG exists in multiple isoforms and it is not known which of these is most important for control of cell viability. Moreover, the levels of cGMP are also controlled by cGMP-phosphodiesterase (PDE) enzymes and the expression of these enzymes has not been characterised in β-cells. In the present study, we have characterised the expression of PKG and cGMP-PDE isoforms in clonal pancreatic β-cells (BRIN-BD11) and have investigated the effects of selective regulators of these enzymes on cell viability.
**Materials and methods:** BRIN-BD11 β-cells were grown in tissue culture and RNA was isolated using RNA-STAT 60 reagent and reverse transcribed prior to PCR amplification. DNA sequencing was performed commercially. Cell death was estimated by flow cytometry and vital dye staining, after exposure to test reagents.
**Results:** Reverse transcription polymerase chain reaction (RT-PCR) analysis revealed the presence of transcripts encoding all three known isoforms of PKG (1α, 1β and II) in BRIN-BD11 cells. In addition, the expression of 2 isoforms of cGMP-PDE, PDE5 and PDE6, were also detected. Confirmation of the identity of these various molecules was confirmed by DNA sequencing. The presence of PDE6 was unexpected since expression of this molecule has been considered to be restricted largely to the visual system. Incubation of β-cells with a selective inhibitor of cGMP-PDE, CP461, caused a dose-dependent loss of cell viability (control: 8.3 ± 1.3% dead cells; 1 μM CP461 – 31.8 ± 2.0%; 5 μM CP461 – 56.9 ± 4.5%; $p<0.001$) suggesting that cGMP-PDE activity may be important for maintenance of viability. Treatment of cells with 2 selective cell permeable activators of PKG-1 also resulted in increased cell death (100 μM – 8-APT-cGMP: 178 ± 36% vs control ($p<0.05$); 100 μM Sp-8-Br-PET-cGMPS – 161 ± 30% vs control; $p<0.05$) and a selective inhibitor of PKG-1 reduced the cell death caused by the guanylyl cyclase activator YC-1 (control: 6.3 ± 0.5% dead cells; 100 μM YC-1: 19.9 ± 1.0%; YC-1 + 1 μM Rp-8-Br-cGMPS: 10.2 ± 1.1% ($p<0.001$). Surprisingly, we also found that exposure of BRIN-BD11 cells to the cytokine, interleukin-4 (IL-4), reduced YC-1-induced death (YC-1: 32.7 ± 6.1% dead cells; YC-1 + IL-4: 17.5 ± 3.7%; $p<0.001$). RT-PCR analysis and DNA sequencing confirmed the expression of receptors for IL-4 in BRIN cells.
**Conclusion:** The results reveal that pancreatic β-cells express multiple PKG and PDE isoforms and demonstrate that regulation of the activity of these enzymes can result in changes in cell viability. The data further imply that sustained activation of PKG-1 may be especially important for control of viability and reveal that the cytokine IL-4 can directly protect β-cells from cell death mediated by agents acting via the cGMP/PKG pathway.
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# Experimental immunology and islet transplantation

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**Regulated gene expression of VEGF-164 by pancreatic beta-cells improves revascularization and engraftment after transplantation**

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**Background:** Revascularization is critical for the adequate endocrine function of transplanted islets of Langerhans. It has been shown that VEGF plays a crucial role in islet neo-vascularisation. The aim of this study was to optimize angiogenesis-enhancing strategies for improving beta-cell engraftment.
**Methods:** CDM3D cells are conditionally immortalized mouse beta-cells modified to overexpress human bcl-2, and can be growth-arrested with tetracycline (TC). They were genetically engineered to secrete mouse VEGF-164, either in a constitutive (PGK cells) or controlled, TC-regulated manner (TET cells). The angiogenic activity of VEGF-secreting CDM3D cells was assessed in vitro using a three-dimensional type I collagen gel model that assays both for endothelial cell invasion and capillary-like tube formation. Three groups of STZ-induced diabetic C3H mice were transplanted under the kidney capsule with cell clusters of either CDM3D, PGK or TET cells. Three other groups of mice were grafted with each cell type (CDM3D, PGK, TET) treated in vitro with TC (1 µg/ml) for 48 hours, and the drug (1 mg/ml) was administered continuously via drinking water to the grafted mice. Two separate groups of mice were transplanted with PGK- and TET-cells and treated with TC only after the glycemia was corrected (BG <11 mmol/l). Time to normoglycemia and intraperitoneal glucose tolerance test (IPGTT) on day 28 after transplantation were assayed.
**Results:** A regulated delivery of VEGF could be achieved in our in-vitro co-culture model. Switching off VEGF gene expression using the TC system resulted in a near complete inhibition of the VEGF-induced angiogenic process. There was a significant difference in the time to return to normoglycemia, when clusters of PGK-cells (19 ± 3 days) and TET-cells (20.5 ± 3.5 days) were transplanted in comparison to non-transduced CDM3D-cells (27 ± 6days). The clearance of plasma glucose during IPGTT was accelerated in PGK-, TET- groups vs. the CDM3D-group ($p<0.01$). TC-treated mice transplanted with TC-treated cells failed to restore normoglycemia and to correct plasma glucose levels on IPGTT.
**Conclusion:** Retroviral transduction of CDM3D-cells with VEGF-164 was highly effective at inducing neo-vessel formation in-vitro and in-vivo and improved blood glucose control in a syngeneic model of islet cell transplantation. Our data demonstrate that overexpression of VEGF has a beneficial effect on beta-cell engraftment.

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**Blood glucose modulates cytokine expression in the initial days after syngeneic islet transplantation**

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**Background and aims:** Transplanted islets are particularly vulnerable in the initial days after transplantation when more than half of transplanted tissue is lost. Non-specific inflammation at the grafted site probably contributes to the initial islet damage. Local production of cytokines could play a role in this initial non-specific inflammation. On the other hand, exposure to hyperglycemia initially after transplantation has a deleterious effect on transplanted islets. The aim of the study was to determine whether the metabolic condition of the recipient modulates cytokine expression in the initial days after islet transplantation.
**Materials and methods:** Male Lewis rats were transplanted with 500 syngeneic islets, an insufficient beta cell mass to restore normoglycemia. Normoglycemic (NG) and hyperglycemic (HG) (streptozotocin-diabetic) rats were used as recipients. Total RNA from freshly isolated islets, 24 h cultured islets, and islet grafts harvested on days 1 and 3 after transplantation was extracted, and reverse transcribed to cDNA. Cytokine expression was determined by real time PCR using a comparative method ($2^{-\Delta\Delta Ct}$ method). mRNA of pro-inflammatory (IL-1β, TNF-α, IFN-γ, and IL-6) and anti-inflammatory (IL-10, and IL-4) cytokines was determined. Results were expressed as fold increase of cytokine expression compared with 24 h cultured islets.
**Results:** In freshly isolated islets IL-1β and TNF-α mRNA were detected at low levels whereas IL-6 and IL-10 transcripts were barely detectable. Low levels of expression of IL-1β, TNF-α, IL-6, and IL-10 were found in 24 h cultured islets, and all they increased strongly on day 1 after transplantation to decrease again on day 3. IL-4 mRNA was not detected in any studied sample. IFN-γ mRNA was not detected in most samples. IL-1β mRNA increased similarly in NG and HG groups on day 1 (NG: 251 ± 93, HG: 228 ± 65) and 3 (NG: 23 ± 3.5, HG: 30 ± 6) after transplantation compared with freshly isolated ($p<0.05$) and cultured islets ($p<0.05$). TNF-α mRNA was also strongly increased on day 1 after transplantation, and was significantly higher in HG (149 ± 23) than NG group (76 ± 19; $p<0.05$) and remained increased in both groups on day 3 after transplantation (NG: 20 ± 4, HG: 33 ± 5) compared with cultured islets ($p<0.05$). IL-6 mRNA was increased on day 1 after transplantation in HG group (16 ± 8; $p<0.02$). IL-10 mRNA was increased in both groups on day 1 after transplantation (NG: 4 ± 0.8, HG: 8 ± 1.5; $p<0.05$) and remained increased in HG group (3 ± 0.5; $p<0.05$) on day 3.
**Conclusion:** The inflammatory response in islet grafts was maximal on day 1 after transplantation and it was still present, although at low levels, on day 3. This non-specific inflammation was enhanced in hyperglycemic recipients that showed higher levels of TNF-α and IL-6 than their normoglycemic counterparts. Deletereous effects of hyperglycemia in islet transplantation may be partly mediated by this increased inflammatory response.
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**Human pancreatic islet rejection during transplantation: the role of chemokine signalling pathway**

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**Background and aims:** During transplantation, pancreatic islets release chemokines promoting macrophage attraction hampering engraftment of islets. The aim of this work was to approach the mechanism of macrophage-pancreatic islets interaction that induces macrophage migration during islet transplantation.
**Materials and methods:** Human monocytic cell line (U937) differentiated into macrophages in presence of phorbol ester meristate (PMA) were exposed to supernatants of human pancreatic islets. Chemotaxis and chemokine receptors expression (CCR-5, CCR-3, CCR-1, CXCR-1) were evaluated. To modulate migration and identify the signalling pathway of macrophage activation, several pharmacological agents were tested: the pertussis toxin to block the Gi protein, staurosporin and wortmannin to inhibit the phosphoinositol-3-kinase and the protein kinase C respectively.
**Results:** In presence of human islet supernatant, macrophage chemotaxis was 3.7 ± 0.5 as compared to 100 pg/ml of CCL-5 (1.4 ± 0.2), to 100 pg/ml of CCL-3 (1.2 ± 0.1) or to 100 pg/ml of CXCL-1 (1.6 ± 0.3). Western blotting analysis of CCR-5 expression showed an increased of CCR-5 expression during the differentiation of monocytic cell line. Furthermore, chemokine receptors expression with pancreatic islet supernatant continues to increase and was dependant on the purity of islets preparation. Finally, chemotaxis analysis with pharmacological agents showed a complete inhibition of macrophage chemotaxis induced by islet supernatant in the presence of pertussis toxin, staurosporin and wortmannin (0,9 ± 0,2, 0,8 ± 0,3 and 1 ± 0,3 respectively versus 3,7 ± 0,5).
**Conclusion:** Blockage of chemokine receptor signalling pathways induce a complete inhibition of macrophage migration suggesting the crucial role of chemokines in islet rejection during transplantation. Moreover, the chemokines receptors CCR-5, CCR-3, CCR-1 and CXCR-1 are involved in islet rejection.

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**Development of a rat model of insulitis by immunisation with 1-Fluoro 2-4 dinitrobenzene (FDNB) tagged rat insulinoma cells: histological and immunological evidence**

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**Background and aims:** Type 1 (IDDM) diabetes has two distinct phases; the first phase is insulitis, which finally progresses to overt diabetes. The nature of the triggering and/or of the target autoantigen(s) is elusive and several candidates have been implicated including insulin, GAD, IA -2 and heat-shock proteins. It was shown previously that chemical manipulation such as tagging of cell surface with 1-fluoro 2,4-dinitrobenzene (FDNB) brings about conformational changes on cell surface. In the present study, immunization of rats with FDNB modified rat insulinoma (RIN) cells produced insulitis.The novelty of the model is explained by further histological and immunological assays.
**Materials and methods:** We immunized groups of rats with either RIN or F-RIN cells. RIN cells were tagged with 1pg of FDNB for 20 min, at room temperature. The cells were washed three times with plain medium to remove untagged FDNB. Each rat received ($1 \times 10^6$) cells four times fortnightly. One month after the last injection, rats were bled to collect sera and sacrificed to collect spleen for antigen specific proliferation assay and pancreas for histology and insulin exhaustion test.
**Results:**
1. Histological analysis of H&E stained formalin fixed pancreas – It was observed that 80% of the F-RIN immunized animal did show 40% or more mononuclear infiltration in the islets (+1 to +4) compared to pancreases from RIN immunized animals.
2. Antibody levels – Circulating IgG2a levels specific to F-RIN cell extract were found to be increased in F-RIN immunized animals indicating Th1-type response in these animals.
3. Pancreatic insulin exhaustion -300 islets each from every RIN and F-RIN immunized rats were challenged with different concentrations of glucose. Islets isolated from F-RIN immunized rats showed significant exhaustion of secretory activity of insulin when challenged with 20 mM glucose and compared with islets isolated from normal and RIN immunized animals.
4. Lymphocyte proliferation assay by $^3$H-thymidine incorporation – Lymphocytes from F-RIN immunized animals showed significant increase in the proliferation in response to irradiated F-RIN cells as compared to irradiated RIN cells.
5. Flow cytometry -RIN immunized sera did not show much reactivity to RIN cells but showed high reactivity to F-RIN cells, whereas F-RIN immunized sera reacted to both RIN as well as F-RIN cells. This suggests FDNB treatment of RIN cells results in exposure of new antigenic sites.
6. Recognition of newer antigens by F-RIN immunized sera in immunoblotting – RIN and F-RIN cell extracts were electrophoresced on SDS-PAGE. The proteins were transferred onto nitrocellulose membrane and exposed to RIN and F-RIN cells immunized rabbit sera. A 26 kDa protein was present in F-RIN and RIN cell extracts recognized by only F-RIN immunized sera and not by RIN immunized sera.
**Conclusion:** Tagging of RIN cells with FDNB brings about conformational changes on RIN cell surface results in exposure of new antigenic sites which on immunization triggers mononuclear infiltration causing insulitis and may probably lead to diabetes.
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**Reduced interleukin 6 production by bone marrow-derived dendritic cells in non-obese diabetic mice**

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**Background and aims:** In type 1 diabetes mellitus, dendritic cells play pivotal roles; they retain capacity to activate autoreactive T cells in the periphery, but are unable to process and/ or present autoantigens in a tolerogenic fashion. Abnormal phenotype and function of DC from NOD mice have been reported. To characterise molecular changes in CD11c$^+$ bone marrow-derived DC from NOD mice, we recently compared the transcript profiles of these cells with those from NON mice. DC from NOD showed 8-fold reduced interleukin 6 (IL-6) mRNA expression compared to those from NON mice. In this study, we examined IL-6 production by DC from NOD mice to confirm the decreased production of this cytokine at protein level. Effect by IL-6 supplementation during differentiation on the phenotype and function of DC was also investigated.
**Materials and methods:** Bone marrow cells from 4-week-old female mice were cultivated in the presence of GM-CSF and IL-4 over 6 days, with or without IL-6 supplementation. CD11c$^+$ DC were sorted by magnetic beads-conjugated anti-CD11c$^+$ antibodies (MACS$^{TM}$).
**Results:** DC from NOD produced significantly lower amount of IL-6 in response to 5 mg/ml LPS (10970 ± 1685.0 pg/ml) than those from NON (45845 ± 3506.6 pg/ml, $p < 0.05$, Mann-Whitney U test). DC from NOD generated with additional IL-6 (2 ng/ml) elicited significantly higher response by CD4$^+$ cells in the syngeneic mixed lymphocyte reaction (SMLR), measured by succinate-tetrazolium reductase activity (WST-1$^{TM}$), than those without IL-6 (optic density 0.36 ± 0.031 vs 0.23 ± 0.027, stimulator: responder ratio = 1:20, $p<0.05$). The up-regulated SMLR was associated with increased median fluorescent intensity of CD80 and CD86 expression by DC (CD80, 116 ± 4.39 vs 92.1 ± 2.75, $p<0.05$; CD86, 13.2 ± 1.50 vs 9.77 ± 0.289, $p<0.05$), and significantly lower IFNg production in the SMLR (412.1 ± 40.94 vs 1639 ± 131.6 pg/ml, $p<0.05$).
**Conclusion:** Reduced autocrine secretion of IL-6 by DC may lead to defective phenotype and function of DC, and then to Th1-deviated immune reaction in NOD mice.
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**Glucose homeostasis in the nonobese diabetic mouse, a spontaneous model of Type 1 diabetes and lymphocyte-deficient NODscid mice during gestation**

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**Background and aims:** We previously reported that, unlike various control strains, nonobese diabetic (NOD) but also NOD*scid* mice, which lack functional lymphocytes and do not develop insulitis and diabetes like NOD mice, experience transient hyperinsulinemia that appears after weaning concomitantly with the first infiltrating macrophages. We also showed transient beta-cell hyperactivity in NOD neonates. We have now studied: 1) the effects of different numbers of successive gestations on diabetes onset in 2 groups of age-matched NOD mothers, and 2) glucose homeostasis during the first gestation in NOD, NOD*scid* and control (C57BL/6) mice.
**Materials and methods:** Various glucose-homeostasis parameters were evaluated, under basal unfasted conditions and after intraperitoneal glucose administration, during gestation I on days 7, 14 and 18 (G7, G14 and G18, respectively) in NOD, NOD*scid* and C57BL/6 mice. The values were also compared to those of strain-matched nongestant mice of similar age (10–12 weeks) and 7-day postpartum dams (D7PP).
**Results:** 1) In aged-matched NOD mothers, 3 vs 2 successive gestations raised diabetes prevalence and mortality; 2) However, during the first and subsequent gestations, unless the mother suddenly became diabetic, NOD maternal glycemia progressively declined as gestation age rose; 3) During gestation I, the basal unfasted glycemia decline was steeper for NOD than C57BL/6 mice; 4) Concomitantly, insulinemia doubled from G7 to D7PP in NOD but not C57BL/6 mice; 5) Of note, glucagonemia tripled in NOD mice from G7 to D7PP, while it increased weakly in C57BL/6; 5) in NOD, as opposed to C57BL6 mice, pancreatic insulin increased sharply from G7 to G18 and fell postnatally, while NOD pancreatic glucagon contents were 2/3 those of C57BL/6 from G7 to D7PP; 6) As expected, under these basal unfasted conditions, blood corticosterone rose progressively to 400–500 ng/ml in both strains, returning to control values D7PP; 7) After glucose injection (1 g/kg) vs NaCl (vehicle), the main result was, on G18, the higher glucose levels in fasted NOD and NOD*scid* mice than C57BL/6, despite higher insulin levels produced in the former 2 strains in response to glucose on G7 and G14. At G18, the NOD insulin response to glucose had fallen sharply compared to G14, suggesting beta-cell exhaustion, probably linked to insulitis progression, unlike in NOD*scid* mice, which showed sustained insulin response to glucose; 8) Corticosteronemia in fasted mice throughout gestation revealed marked strain differences: while they rose progressively in C57BL/6 mice, reaching 4000 ng/ml on G18, NOD and NOD*scid* values peaked on G14 (respectively, 6000 and 8000 ng/ml), followed by steep declines thereafter.
**Conclusion:** Beta-cell hyperactivity is a characteristic of adult nonpregnant and pregnant NOD and NOD*scid* mice. During gestation, this hyperactivity is intensified and associated with deregulation of glucagon and corticosterone secretion. Notably, the strongly hyperactive hypothalamo-pituitary-adrenal axis might worsen insulin resistance during late gestation, further exhausting beta cells and affecting fetal development.
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**Mice deficient in the OGG-1 DNA glycosylase are protected from multiple-low-dose-streptozotocin-induced diabetes**

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**Background and aim:** OGG-1 DNA glycosylase is an enzyme involved in DNA repair which excises 7,8-dihydro-8-oxoguanine, which is formed by oxidative damage of guanine at the 8 position. The DNA repair enzyme poly (ADP-ribose) synthetase has been demonstrated to have other cellular effects as well as DNA repair including modulation of the immune system. The aim of this study was to examine the role of OGG-1 in Type I diabetes. To this end we utilised transgenic mice with the OGG-1 gene disrupted and the multiple-low-dose-streptozotocin (MLDS) model of Type I diabetes. MLDS treatment of mice induces insulitis and progressive hyperglycemia.
**Materials and methods:** Male, age-matched mice of the OGG-/- and OGG+/+ phenotype were treated with streptozotocin (stz). Diabetes was induced by IP injection of stz (40 mg/kg) or vehicle (citrate buffer) on five consecutive days, blood glucose was monitored over a 21-day period after the first stz injection. Pancreas samples were taken on day 21 and total insulin extracted and expressed as ng insulin/mg protein; in addition pancreatic cytokine and chemokine levels were determined.
**Results:** The stz treated OGG+/+ mice progressively developed hyperglycemia, blood glucose levels of $101 \pm 2$ and $262 \pm 18$ mg/dl ($n=20$, $p<0.01$) on day 1 and 21 respectively, with 80% of the mice diabetic (blood glucose > 200 mg/dl) on day 21. In the OGG-/- mice there was only a minimal increase in blood glucose levels from $109 \pm 3$ to $136 \pm 8$ mg/dl ($n=20$, $p<0.05$) and only 10% incidence of diabetes on day 21. Stz treatment decreased pancreas insulin content from $76 \pm 4$ to $14 \pm 2$ ng insulin/mg protein ($n=10$, $P<0.01$) in the OGG+/+ mice; the stz treated OGG-/- mice had a significantly higher insulin content $36 \pm 3$ ng insulin/mg protein ($n=10$, $p<0.01$). In addition pancreas protein levels of the chemokine, MIP-1α ($0.6 \pm 0.1$ to $1.4 \pm 0.3$ pg/mg protein; $n=10$, $p<0.01$), and the inflammatory cytokines, TNF-α ($4.7 \pm 0.9$ to $12.5 \pm$ pg/mg protein;3; $n=10$, $p<0.01$) and IL-12 (p40) ($15 \pm 2$ to $37 \pm 4$ pg/mg protein; $n=10$, $p<0.01$), were all increased in OGG+/+ mice following MLDS treatment whereas the levels of MIP-1α ($0.8 \pm 0.1$ pg/mg protein), TNF-α ($5.1 \pm 1$ pg/mg protein) and IL-12 (p40) ($19 \pm 1$ pg/mg protein) were all significantly lower in the pancreas of MLDS-treated OGG-/-. MLDS had no effect on the levels of Th2 cytokines in the pancreas of OGG-1+/+ mice but both IL-4 and IL-10 levels were significantly higher in the pancreas of OGG-1-/- mice.
**Conclusion:** OGG-1 deficient mice are protected from MLDS-induced Type I diabetes. Previously we have shown OGG-1 deficient mice to be resistant to inflammation induced by both lipopolysaccharide-mediated shock and oxazolone-mediated contact hypersensitivity, we have now determined that OGG-1 mice are also resistant to autoimmune diseases. This protection from diabetes by OGG-1 gene disruption is associated with a marked decrease in cytokine and chemokine production. Therefore, we propose that OGG-1, in addition to being a DNA repair enzyme, also functions as an inflammatory/immune system modulator.
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# PS 30

# Prediction and prevention of Type 1 diabetes mellitus

## 500

**Association of enterovirus infections and autoantibody defined risk to develop Type 1 diabetes in schoolchildren from the general population**

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**Background and aims:** Enterovirus (EV) infections have been considered a possible environmental contributor to the manifestation of type 1 diabetes (T1D) in several but not all studies performed so far. As shown for autoantibody (AAb) positive first degree relatives of patients, the risk to develop T1D is also increased in probands from the general population if multiple AAbs against different beta cell antigens occur. The aim of the study was to evaluate if EV infections are associated with the risk to develop T1D in schoolchildren determined by general population based AAb screening.
**Materials and methods:** Fifty healthy probands without heredity of T1D recruited from the Karlsburg type 1 diabetes risk study were differentiated according to their AAbs into three risk groups: 29 probands at high risk (HR; more than 1 AAb), 10 at moderate risk (MR; single AAbs at high titer) and 11 at low risk (LR; single AAbs at low titer). Furthermore, 50 AAb negative control children were involved. AAbs against GAD65 (GADA), protein tyrosin phosphatase (IA-2A), insulin (IAA) were detected by 125I-antigen binding assays at or above the 99$^{th}$ percentile and AAbs against islet cell antigens (ICA) immunohistochemically at or above 20 JDFunits. HLA-DQB1 alleles were defined by DNA genotyping by PCR. EV-RNA sequences were detected by PCR in sera of AAb-positive and AAb-negative children.
**Results:** Among all three risk groups GADA occur most frequently, whereas in children at MR and LR the IA-2A and ICA occur at lowest frequency. The AAb defined risk reveals also a differentiated genetic susceptibility, as frequencies of the diabetes-associated alleles *02 and/or *0302 were significantly enhanced and the protective allele *0602 was diminished in HR probands in comparison to the LR group ($p<0.01$). The overall prevalence of EV-RNA in AAb positive children was 20% (10/50) and significantly increased comparison to controls (4%; 2/50). Using real-time PCR the prevalence improved to 34% (17/50) and 14% (7/50), respectively ($p<0.05$). However, if the prevalence of EV-RNA is analyzed according to AAb defined risk, only children at HR have significantly increased frequencies of virus infections in comparison to the control group: 24.1% (7/29, $p=0.02$) in RT-PCR and 44.8% (13/29, $p=0.006$) in real-time PCR. The prevalence of EV-RNA in children at MR or LR did not differ from those of controls.
**Conclusion:** Our data demonstrate that EV infections are a risk factor for T1D and are associated with the induction of beta cell autoimmunity before the clinical onset of the disease. Especially in genetically susceptible probands at high risk identified by combined AAb screening from the general population, EV infections seems to accelerate and/or initiate the process of beta cell destruction.

## 501

**Simultaneous detection of autoantibodies to GAD65 and IA-2 in children with genetic risk for Type 1 diabetes by time-resolved immunofluorometric dual label assay**

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**Background and aims:** The presence of circulating autoantibodies to beta-cell antigens, such as glutamic acid decarboxylase (GAD65) and protein tyrosine phosphatase-like molecule IA-2, is a predictive sign of Type 1 diabetes. The aim of the study was to develop a novel dual label time-resolved immunofluorometric assay (TR-IFMA) for simultaneous detection of autoantibodies to GAD65 (GADA) and IA-2 (IA-2A), using the Wallac DELFIA® Immunoassay System. We also evaluated the clinical performance of the method in screening serum samples from a cohort of children

at genetic risk for Type 1 diabetes and compared the results to those obtained by separate, single label assays.
**Materials and methods:** Serum samples from a cohort of 100 children at genetic risk for Type 1 diabetes and from 100 healthy control children were analysed for the presence of GADA and IA-2A.
Biotinylated GAD65 and IA-2A proteins, GST-IA-2 fusion protein, europium-labelled GAD65, terbium-labelled anti-GST antibody and serum sample or calibrator were incubated in a casein-containing Delfia Assay buffer in 1.5 ml eppendorf tubes for 45 min with continuous shaking on a shaker. Aliquots were transferred to the wells of streptavidin-coated 96-well microtiter plates and incubation was continued for 30 min. The wells were washed, Delfia Enhancement solution was added to the wells and the plate was shaken for 15 min. The fluorescence of europium was measured in a Delfia 1234 Plate Fluorometer. Delfia Terbium Enhancement solution was added to each well, the plate was shaken for 5 min and allowed to equilibrate. Terbium fluorescence signal was measured and Wallac Multicalc program was used for the calculation of the results. Total performance time for one plate (40 samples) was 2.5 hours. The single label GADA and IA-2A TR-IFMA assays were performed on streptavidin coated 96-well microtiter plates as described by us previously. All reagents were from PerkinElmer Life and Analytical Sciences Wallac Oy, Turku, Finland.
**Results:** 64 of the 100 children at genetic risk for Type 1 diabetes were GADA positive in dual assay and 65 in single label GADA assay. Both methods detected 63 of them, one child was positive in dual assay only and two children were positive in single assay only. All the discrepant cases had low GADA concentrations, close to cut-off limits. 73 of the 100 children at genetic risk were found IA-2A positive in dual assay and all of them were detected positive also in the single label IA-2A assay. The numerical correlation between the results obtained by dual and single label assays was good ($r = 0.962$ for GADA and $r = 0.836$ for IA-2A).
**Conclusion:** The performance of the dual label TR-IFMA for GADA and IA-2A compared excellently with separate, single label GADA and IA-2A TR-IFMA assays developed recently in our laboratory. The new method proved simple and rapid to perform, and can easily be adapted to automated platforms allowing further significant reduction in performance time and labour costs.
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## 502

**Immunologic phenotype and ultrastructure of lymphocytes in normoglycemic first-degree relatives of patients with Type 1 diabetes positive for diabetes-associated autoantibodies**
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**Background and aims:** The onset of type 1 diabetes mellitus is characterized by immune aggression toward pancreatic beta-cells. However, the immunologic phenotype and ultractructure of lymphocytes in subjects with preclinical stage of diabetes remains not known. The aim of our study was to investigate immunophenotype and ultrastructure of lymphocytes in normoglycemic first-degree relatives of patients with type 1 diabetes with or without the presence of diabetes-associated autoantibodies.
**Materials and methods:** We examined 120 children with normoglycemia aged 8–15 years old – 70 first-degree relatives of patients with type 1 diabetes (35 positive for DAA (DAA+), 35 negative for those antibodies (DAA-) and 50 healthy control subjects without family history of type 1 diabetes and no presence of DAA. The titers of autoantibodies to GAD, insulin and tyrosine phosphatase were measured by radioimmune assay. The children were considered to be DAA+ in case of elevated titer of 2 or more DAA. Immunophenotype of lymphocytes (CD3+, CD4+, CD8+, CD20+ and CD56+ cells) was analyzed by fluorescence-activated flow cytometry. The ultrastructure of lymphocytes was studied with electron microscopy. Statistical analysis was performed by Student's test.
**Results:** We found significant decrease of the number of CD3+ and CD4+ cells and CD4/CD8 ratio in DAA+ group compared to DAA- or control groups. The number of CD3+ cells was $0.49 \pm 0.05 \cdot 10^9$/l, $1.23 \pm 0.05 \cdot 10^9$/l, and $1.29 \pm 0.07 \cdot 10^9$/l; the number of CD4+ cells was $0.49 \pm 0.04 \cdot 10^9$/l, $0.81 \pm 0.05 \cdot 10^9$/l, $0.88 \pm 0.05 \cdot 10^9$/l, and CD4/CD8 ratio was $1.7 \pm 0.1$, $1.9 \pm 0.1$, $2.1 \pm 0.1$ in DAA+, DAA- and control groups, respectively, $p<0.05$ for comparison of DAA+ group with two others. In DAA+ subjects the changes of ultastructure of CD4+ cells, especially in Goll's bodies indicating an enhanced functional activity were noted.
**Conclusion:** The revealed changes of immunologic phenotype and ultrastructure of lymphocytes in the first-degree normoglycemic DAA+ relatives of diabetic patients could indicate that the disturbances of T-cells lymphocytes occur at the very early stages of the pathogenesis of type 1 diabetes mellitus.

## 503

**Characterization of autoreactive and induced anti-insulin antibody epitopes in man and mouse**
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**Background and aims:** Insulin autoantibodies are a marker of pre-clinical diabetes, and insulin treatment promotes the production of insulin antibodies. The aim of this study was to determine whether there are differences in insulin binding in insulin autoantibodies and insulin antibodies and whether the differences relate to diabetes risk or the mode of immunisation.
**Materials and methods:** Insulin binding to $^{125}$I-human insulin was competed with increasing concentrations of species specific insulins (human, rat, chicken) and insulin analogues (ovine (A8 his), human (B28 lys, B29 pro), human (A13 trp, B28 lys B29 pro)) using a protein A/G radiobinding assay. Binding was analysed in insulin autoantibody positive first degree relatives and in female NOD mice, in insulin treated patients with type 1 diabetes, type 2 diabetes and gestational diabetes, and insulin immunised NOD mice and BALB/c mice.
**Results:** Heterogeneity between IAA was observed with respect to the relative inhibitory capacities of competing insulins. One group of relatives had high binding affinity to human insulin (KD, $>10^9$), and were similarly inhibited by human (B28 lys, B29 pro) insulin analogue (median EC50 relative to human insulin, 1.2 fold), had lesser inhibition with ovine (A8 his) insulin analogue (7 fold less than human) and rat insulin (15 fold less than human) and minimal inhibition with chicken insulin (62 fold less than human) and the human (A13 trp, B28 lys, B29 pro) insulin analogue (49 fold less than human). A second group of relatives had IAA of lower affinity to human insulin (KD, $10^6$–$10^9$) and these had markedly different inhibition profiles that were most consistently distinguished by absent binding to the human (B28 lys, B29 pro) insulin analogue. Relatives with the high affinity type IAA had multiple islet autoantibodies and high diabetes risk whereas the low affinity type IAA did not. Antibodies against insulin in insulin treated patients were of high affinity ($>10^9$) except in one patient with type 2 diabetes, and had inhibition patterns that were similar, but not identical to the high affinity IAA. Untreated NOD mice had IAA with high affinity to human and rat insulin. In comparison, human insulin immunized NOD mice had antibodies that bound both rat and human insulin with higher affinity to human than rat insulin, whereas human insulin immunized Balb/c mice had insulin antibodies that bound strongly to human insulin and only very weakly to rat insulin.
**Conclusion:** The C-terminal end of the insulin B chain potentially distinguishes IAA with different affinity and diabetes risk. Lower affinity low risk IAA appear to bind an epitope that requires the correct sequence and conformation of the C-terminal of insulin B chain that is important for insulin dimer formation, whereas the diabetes relevant high affinity IAA are unaffected by changes in this region of the B chain, but require correct sequence and conformation of the insulin A chain.
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## 504

**The 6.1kb 3'-region of the CTLA4 gene polymorphism is associated with susceptibility to fulminant Type 1 diabetes**
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**Background and aims:** Fulminant Type 1 diabetes is a novel subgroup of Type 1 diabetes characterized by the remarkably rapid onset with near-normal HbA1c levels, high frequency of flu-like symptoms before onset, exhausted insulin-secretory capacity at onset, negative for anti-islet autoantibodies, and T-cell infiltration of the exocrine pancreas. The etiology of fulminant Type 1 diabetes has not been solved whether anti-islet autoimmunity is associated with the development of the disease. The CTLA4

gene has been known to encode the T-cell receptor responsible for T-cell proliferation and apoptosis, and has been reported to be a strong candidate for T-cell mediated autoimmune disorders including Type 1 diabetes. The aim of this study was to examine the possible association between the CTLA4 gene polymorphisms and the susceptibility to fulminant Type 1 diabetes.
**Materials and methods:** Fifty-five patients with fulminant type 1 diabetes, 90 acute-onset patients with Type 1 diabetes, and 117 healthy control subjects were examined the A/G polymorphism at position 49 and the recently identified A/G polymorphism at 6.1 kb 3'-region (CT60) of the CTLA4 gene using PCR-RFLP methods. Statistical analyses were performed by Chi-square test.
**Results:** The genotype distribution and allele frequencies of the +49 A/G polymorphism differed significantly between patients with acute-onset Type 1 diabetes and healthy controls. The G allele frequency in acute-onset patients with Type 1 diabetes (73%) was significantly higher than that in controls (56%, p=0.0004). The CT60 polymorphism was not associated with acute-onset type 1 diabetes. In contrast, the genotypic distribution and allele frequencies of CT60 polymorphism in patients with fulminant Type 1 diabetes differed significantly from those in healthy controls ($p < 0.05$). The presence of AA genotype was associated with an increased risk of fulminant Type 1 diabetes (OR=3.8, 95%CI=1.2–12.3; p=0.018). However, the CTLA4 +49 A/G polymorphism was not associated with the genetic susceptibility to fulminant Type 1 diabetes.
**Conclusion:** These results suggest that 6.1 kb 3'-region A/G polymorphism in the CTLA4 gene confers an increased genetic susceptibility to fulminant Type 1 diabetes. Furthermore, genetic contribution of the two CTLA4 gene polymorphism to disease susceptibility differs between fulminant Type 1 diabetes and classical acute-onset Type 1 diabetes.

## 505

### Soluble interleukin-2 receptor and neopterin in sera of patients with newly diagnosed Type 1 diabetes and prediabetes

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**Background and aims:** Soluble interleukin-2 receptor (sIL-2R) is produced by activated T and B cells, and the level of this receptor is elevated in patients with some autoimmune diseases, allergic inflammation and some neoplasms. Neopterin is a marker associated with cell-mediated immunity as well. It is produced by monocytes/macrophages primarily upon stimulation with interferon-gamma. Increased neopterin production is found in virus infections, intracellular living bacteria and parasites infections, autoimmune diseases, malignant tumor diseases and in allograft rejection episodes. This study have demonstrated role of this substances in type 1 diabetes.
**Materials and methods:** The concentration of sIL-2R and neopterin was determined by enzyme-linked immunosorbent assay (ELISA). We studied 29 patients with type 1 diabetes, 30 patients with prediabetes and 30 healthy controls.
**Results:** Serum sIL-2R levels in prediabetes were significantly higher than those in healthy controls [0,174 ± 0,25 ng/ml vs. 0,048 ± 0,039 ng/ml; P = 0.04]. Mean sIL-2R levels were, however, similar in diabetic and controls (0,095 ± 0,18 ng/ml vs. 0,048 ± 0,039 ng/ml; P=NS) and in diabetic and prediabetes (0,095 ± 0,18 ng/ml vs. 0,174 ± 0,25 ng/ml; P=NS). Conversely lower levels of neopterin were found in the sera of prediabetic patients compared with diabetic patients [4,73 ± 5,22 nmol/l vs. 8,90 ± 2,1nmol/l; P=0,04]. Levels of serum neopterin were not significantly different in diabetes and healthy controls [8,90 ± 2,1nmol/l vs. 8,92 ± 5,22nmol/l; P=NS].
**Conclusion:** Our observations suggest that serum sIL-2R and neopterin is a good measure of the diabetes activity and could be useful markers to monitoring the course of prediabetes
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## 506

### 1,25-Dihydroxy-Vitamin D3 for preservation of beta cell function in patients with newly diagnosed Type 1 diabetes

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**Background and aims:** $1,25(OH)_2D3$ (VitD3) treatment, the activated form of vitamin D, has important immunomodulatory effects. We have previously reported that $1,25(OH)_2D3$ (VitD3) in a dose of 0.25 µg/day is a safe and tolerable therapy in patients with newly diagnosed T1D (IDS Paris, 2003). In NOD mice, prevention of autoimmune diabetes and insulitis can be very efficiently achieved using VitD3 treatment from 3 weeks of age. We now investigated the effect of VitD3 on β-cell function in a randomized open pilot trial in patients with newly diagnosed type 1 diabetes (T1D).
**Materials and methods:** Of patients eligible for enrollment (inclusion criteria: age 18–39 years, <14 days insulin therapy, GADA and/or IA2A pos.), 18 were randomized to daily VitD3 supplementation (0.25 µg Rocaltrol®) and 10 served as controls (median age treated 27 years vs controls 32 years). Treated patients received VitD3 for 9 months followed by 9 months observation without supplementation (median follow-up 18 months (range 8–18 months).
**Results:** No difference between HbA1 c, BMI, and insulin requirement, fasting BG, fasting and stimulated C-peptide as well as delta AUC was observed between patients with and without supplementation.
**Conclusion:** We conclude that within this pilot trial $1,25(OH)_2D3$ supplementation does not show a significant benefit to beta cell function and metabolic control compared to intensive insulin therapy in patients with newly diagnosed T1D.

| Variable | Follow-up (months) after randomization in the study | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 (baseline) VitD3 treated (n=18) Median values | 1 | 9 | 18 | 0 (baseline) Controls (n=10) Median values | 1 | 9 | 18 |
| HbA1 c (%) | 10.4 | 8.0 | 6.4 | 7.2 | 12.6 | 8.2 | 6.0 | 6.2 |
| BMI (kg/m²) | 21.9 | | 23 | 24.2 | 22.4 | | 22.5 | 22.9 |
| Insulin /KG BW (I.U./kg) | 0.48 | 0.33 | 0.37 | 0.45 | 0.59 | 0.37 | 0.4 | 0.44 |
| Fasting BG (mg/dl) | | | 116 | 148 | | | 100 | 136 |
| Fasting C-peptide (ng/ml) | 0.8 | 0.96 | 1.04 | | | 0.58 | 0.4 | 0.4 |
| AUC (ng/ml/120 min) | 203 | 241 | 227 | | | 154 | 149 | 119 |
| Delta AUC | | 24 | 58 | | | | 20 | −47 |

AUC = area under the curve, delta AUC = 9 months or 18 months AUC minus 1 month AUC, BMI =body mass index, BG = blood glucose, KG BW = kilogram body weight.

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## 507

### Calcitriol versus nicotinamide in patients with recent onset Type 1 diabetes: IMDIAB XI randomised trial

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**Background and aims:** A number of recent studies underline the importance of vitamin D in the pathogenesis of type 1 diabetes (T1D). The main findings include: (a) supplementation with vitamin D (vit D) at birth reduced the incidence of T1D later in life; (b) epidemiological data from the EURODIAB study showed that vitamin D supplementation has protective effects on T1D; (c) recent data from our group identified a reduction of $1,25(OH_2)$ vitamin $D_3$ (the active form) in patients with recent onset T1D. The aim of this study was to evaluate whether supplementation with the active form of vitamin D (calcitriol) at diagnosis of recent onset T1D can favour clinical remission and improve the integrated parameters of metabolic control (HbA1 c, insulin requirement and C-peptide secretion).
**Materials and methods:** In this IMDIAB 11 randomised trial, a total of 76 patients with recent onset (<4 weeks) T1D, mean age 12.8 years ± 7.6 SD were enrolled. Patients were randomised either to calcitriol (0.25 ug/day on separate days) or nicotinamide (NA) 25 mg/kg daily. In both groups of patients, intensive insulin therapy was implemented with three administrations of regular insulin daily + NPH insulin at bed time. Frequent telephone consultations (at least once per week) were arranged with the enrolled patients.
**Results:** We report now results 1 year after diagnosis. In both groups of enrolled patients HbA1 c and insulin requirement dropped significantly after 3 months of therapy. In both groups mean HbA1 c levels were below 7% throughout the first year of the disease. Baseline C-peptide values were not different between the two groups at diagnosis and at 1 year, and did not

drop at 1 year compared to diagnosis in both groups. Most interestingly however, C-peptide values following stimulated test with Sustacal were significantly higher in the calcitriol treated group vs.the NA group ($p<0.03$). Finally, in the calcitriol group treated, calcium levels remained within normal ranges and no adverse effects were noted.
**Conclusion:** This the first study that investigated the effects of calcitriol at diagnosis of T1D and compared it to NA. The preliminary data reported here is encouraging as it indicates that calcitriol may be beneficial in increasing residual beta cell function without inducing hypercalcemia at the dose used in the present trial.

# PS 31
# Clinical immunology

## 508

**TNF-α, TGF-β1, IL-10, IL-6, gene polymorphisms in latent autoimmune diabetes in adults (LADA) and Type 2 diabetes mellitus**

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**Background and aims:** Abundant evidence suggests that cytokines play an important role in the pathogenesis of autoimmune diabetes, such as latent autoimmune diabetes in adults (LADA).
The aim of the present study was an attempt to investigate cytokine polymorphic genes and to correlate them with the likelihood of developing diabetes mellitus and more specifically, type 2 and LADA diabetes.
**Materials and methods:** A total of 64 Greek diabetic patients, 32 patients with LADA diabetes, 32 patients with type 2 diabetes and 39 healthy control subjects were evaluated. We investigated IL-6-174G/C, TNF-α-308A/G, TGF-β1-codon 10 T/C, TGF-β1-codon 25 G/C, IL-10-1082 A/G, IL-10-819 T/C, IL-10-592 A/C gene polymorphisms in all study groups. The diagnosis of diabetes type was based on the presence of GAD-65 Abs.
Genotypic analysis was performed by using the sequence specific primer polymerase chain reaction (PCR-SSP) methodology. Phenotypic, genotypic and allelic frequencies were evaluated by chi-square analysis with $2 \times 2$ or $2 \times n$ contingency tables.
**Results:** A significant difference in the frequencies of 1082 A/G IL-10 alleles was observed between LADA and diabetes type 2 patient groups. Carriers of the +1082 A allele, which is known to be associated with low IL-10 production, were more frequent among LADA diabetics (93,8%) than type 2 diabetics (74,2%) ($p=0.036$, OR=5.217).No significant differences of genotypes, phenotypes or allele frequencies in the remaining cytokine polymorphisms were observed between the study groups. Analysis of allele combinations revealed a significant involvement of the low and high in vitro production IL-10 alleles in the development of LADA and type 2 diabetes, respectively.
**Conclusion:** These results suggest that the G/A polymorphism at position +1082 of IL-10 promoter gene region may contribute to the pathogenesis of LADA diabetes. Identification of cytokine gene polymorphisms may establish new biomarkers leading to early and definite diagnosis of the different types of diabetes mellitus.

## 509

**GAD65 autoantibody titres at diagnosis in Latent Autoimmune Diabetes in Adults (LADA) differ from Type 1 diabetes (T1D) and together with epitope specificity predict insulin requirement**

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**Background and aims:** Latent Autoimmune Diabetes in Adults (LADA) is a slowly-progressing form of autoimmune diabetes characterised by autoantibodies against glutamic acid decarboxylase (GAD65) in subjects with a clinical diagnosis of Type 2 diabetes (T2D). It is unclear whether there are differences in the autoimmune response to GAD65 between LADA and Type 1 diabetes (T1D). Our aims were: (i) To compare titres of GAD65 autoantibodies (GADA) in LADA patients from the UK Prospective Diabetes Study (UKPDS) cohort with those in T1D patients from the Bart's-Oxford (BOX) Study. (ii) To relate titre and epitope specificity of GADA in LADA to clinical phenotype.
**Materials and methods:** GADA titres at diagnosis (defined as 'Low', 'Medium' and 'High' according to titres in T1D) were determined by radioimmunoassay in 282 LADA subjects (median age 47yr [25–65]) and 689 GADA-positive children with T1D (median age 11yr [0.75–21]). Within the LADA cohort, GADA titre was related to clinical phenotype (age at

onset, BMI, β-cell function and time to insulin requirement). GAD65/67 fusion proteins were used to determine immunoreactivity towards N-terminal (N), middle region (M) and C-terminal (C) epitopes of GAD65 in the LADA group.
**Results:** More patients with LADA than T1D had higher GADA titres; Low: 12% vs 33%, Medium: 29% vs 30%, High: 59% vs 37%, respectively (trend-test, p<0.0001). Among LADA patients, requirement for insulin therapy within 6 years of diagnosis correlated with GADA titre: lower third 62%, middle third 70% and upper third 78% (trend-test p=0.039). Higher GADA titres correlated also with immunoreactivity to multiple GAD65 epitopes at diagnosis. The proportion of patients immunoreactive to N : M : C epitopes by titre third were; lower third 21% : 68% : 53%, middle third 68% : 92% : 88%, upper third 89% : 97% : 96%, respectively (trend-tests p<0.0001). Reactivity to N-terminal epitopes in LADA was a significant predictor of insulin requirement by 6 yrs (p=0.015). Neither overall GAD65 antibody titre nor epitope positivity were associated with age at onset, BMI or %BHOMA.
**Conclusion:** Within the autoimmune diabetes spectrum, higher GADA titres associate with latent onset and a slowly-progressing disease process. However, within LADA, increasing GADA titre and N-terminal epitope reactivity at diagnosis increase the likelihood for future insulin requirement.
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**Different TH1/TH2 cytokine expression in Type 1 diabetes mellitus patients (T1DM) alone or associated with autoimmune thyroid disease (AITD)**
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T1DM and AITD represent two different diseases with the tendency to be clinically associated and they are both characterized by circulating auto-antibodies and intraglandular lymphocyte infiltration. Patients with T1DM, a typical TH1 phenotype disease, are often affected with AITD, either Hashimoto's Thyroiditis (HT), which is characterized by the same cytokine pattern, or Graves Disease (GD), which instead is characterized by a TH2 cytokine phenotype. The aim of the study was to evaluate cytokine profile in patients with T1DM, T1DM1/HT, T1DM/GD and GD.
T1DM patients, examined in good metabolic control (HbA1 c < 7%), were treated with basal-bolus insulin therapy, T1DM/HT patients with replacement LT-4 therapy and T1DM/GD patients with methimazole. TH1 (IFNγ and IL-2) and TH2 (IL-4 and IL-13) cytokine expression was evaluated both on resting and activated peripheral blood lymphocytes [Ionomicin (1 μg/ml) + PMA (25 ng/ml), previously treated with brefeldin (10 μg/ml)]. T cells were stained with FITC or PE-conjugated surface antibodies (anti-CD4, anti-CD8), then permeabilized and treated with anti-cytokine fluorescent-conjugated intracellular antibodies (anti-IL4, anti-IL13, anti-IFNγ, anti-IL-2) and analysed by flow cytometric analysis. A high expression of TH1 cytokines was found in T1DM patients (IFNγ: 64.7 +/- 29.5%; IL-2: 52.8 +/- 16%) when compared to T1DM/GD (IFNγ: 18.4 +/- 2.2%; IL-2: 11 +/- 3%, p<0.001), T1DM/HT (IFNγ: 38 +/- 15%; IL-2: 32 +/- 10%; p<0.01) and GD (IFNγ: 22 +/- 14%; IL-2: 25.3 +/- 13.5%, p<0.001)). T1DM/GD patients showed a high TH2 phenotype expression (IL-4: 48.7 +/- 5.2%; IL-13: 39.5 +/- 2.1%) when compared to both T1DM (IL-4: 20 +/- 5.3%; IL-13: 14.2 +/- 4.7%; p<0.001) and T1DM/HT patients (IL-4: 18.4 +/- 3.0%; IL-13: 14.4 +/- 5.1%; p<0.001). GD patients showed a TH2 phenotype similar to T1DM/GD patients, although expressed at a lower level (IL-4: 37.7 +/- 15%; IL-13: 28.3 +/- 12%, p: NS). The different cytokine profile found in diabetic patients with associated AITD reveals how a classic TH1 disease can shift to a TH2 disease, as in T1DM/GD. We suggest that cytokine expression analysis may be a useful marker to identify T1DM candidates at high risk for another associated autoimmune disorder.
*We would like to thank Diabetes UK for funding this work.*

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**Monocytes from Type 2 diabetic patients have pro-inflammatory cytokine profile**
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**Background and aims:** An inflammatory component is believed to play a role in the development of Type 2 Diabetes (T2D) and in particular of its complications. As source of inflammatory products such as CRP and cytokines, mainly liver and endothelial cells have been studied. The aim of this work was to assess the cytokine and chemokine profile of macrophages from T2D patients compared to healthy controls and type 1 diabetic patients (T1D) and to evaluate the anti-inflammatory capacity of 1,25dyhydroxyvitamin $D_3$ ($1,25D_3$; the active form of vitamin D).
**Materials and methods:** CD14 positive monocytes were isolated from peripheral blood mononuclear cells with MACS technology. Cells were cultured for 3, 6, 9, 12, 24 or 48 hs in the presence of either IFNγ alone or IFNγ plus $1,25D_3$. Cells at the end of each time point were harvested and real-time RT-PCR was performed for gene expression determination.
**Results:** Monocytes from T2D patients presented higher expression of TNFα already at basal level (p<0.01) when compared to controls. After IFNγ stimulation a ten-fold higher induction of IL1 and TNFα expression was seen (p<0.01). ICAM and IL8 were also highly expressed by monocytes of T2D patients compared to controls and T1D patients (8-fold, p<0.01). Preliminary data in $PGS_2$, IL6, IP-10 and MCP-1 also have shown an aberrant expression profile in these patients. $1,25D_3$ incubation was able to diminish expression of TNFα and IL1 (2-fold). Moreover, pre-incubation with $1,25D_3$ for 18 hs prior to IFN stimulation was able to complete abrogate IL1, TNFα and IL6 expression.
**Conclusion:** We conclude that monocytes from type 2 diabetic patients might be an important source of inflammatory cytokines, possible playing a role in the pathogenesis of cardiovascular complications and the disease itself. $1,25D_3$ is a potent down-regulator of this inflammatory profile.

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**Intracytoplasmic cytokine levels and neutrophil functions in early clinical stage of Type 1 diabetes mellitus with/without insulin treatment**
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**Background and aims:** Many studies investigated the role of Th1 and Th2 cytokines in Type 1 diabetes, there is no study about the cytokine profile in different clinical stages of the disease. In-vitro studies indicate disturbances of oxidative burst and phagocytic activity in neutrophils of diabetic patients with uncontrolled disease. These studies support the importance of neutrophil functions in the treatment and follow-up of diabetic patients.
**Materials and methods:** Patients diagnosed as Type 1 diabetes but not yet taken under insulin therapy (Group 1, n:10, mean age: 32.4 ± 10) and Type 1 diabetes patients with disease duration of < 3 months (Group 2, n:10, mean age: 25 ± 7.5) were compared to healthy control subjects (Group 3, n:10, mean age: 35.6 ± 8). ICA and GADA were determined by indirect immunofluoresce and ELISA respectively. In isolated $CD4^+$ and $CD8^+$ T cells by MACS technology, intracytoplasmic IL-2, IL-10, IFN-γ and TNF-α levels and neutrophil functions were determined by Flow Cytometry.
**Results:** TNF-α of $CD8^+$ T cells compared to $CD4^+$ T cells and IL-2 of $CD4^+$ T cells compared to $CD8^+$ T cells found to be higher in Group 1 (p<0.001). IL-10 and IFN-γ did not show any difference in $CD4^+$ and $CD8^+$ T cells (p<0.05). TNF-α and IFN-γ of $CD8^+$ T cells compared to $CD4^+$ T cells and IL-2 of $CD4^+$ T cells compared to $CD8^+$ T cells were higher in Group 2 (p<0.05 and p<0.01, respectively). TNF-α and IL-2 were found to be higher in $CD4^+$ T cells compared to $CD8^+$ T cells in Group 3 (p<0.01 and p<0.001, respectively). IL-10 was also higher in $CD4^+$ T cells compared to $CD8^+$ T cells (p<0.05).
Evaluating Group 1 and Group 2 generally, in spite of the increase of TNF-α, IL-2 and IL-10 in $CD4^+$ T cells, IFN-γ was higher in $CD8^+$ T cells compared to $CD4^+$ T cells (p<0.01).
Verifying cytokines of CD4+ T cells in all three groups, no difference was observed in
TNF-α in Group 2 compared to Group 3 (p>0.05), but a statistically significant decrease was found in Group 1 compared to Group 2 and Group 3 (p<0.001). IL-2 was decreased significantly in Group 1 and Group 2 compared to controls (Group 3) (p<0.001 and p<0.01, respectively). Comparing IFN-γ; no difference was observed in Group 1 and Group 3 (p>0.05), but IFN-γ of CD4+ T cells were significantly lower in Group 2 compared to other groups (p<0.001).Comparing cytokines of $CD8^+$ T cells in all groups, TNF-α was higher in Group 1 compared to Group 2 (p<0.05), and also higher in Group 1 and 2 compared to Group 3 (p<0.001). IL-2 was increased in Group 1 compared to Group 2 (p<0.05), but the levels in both groups were lower than Group 3 (p<0.001). Although IFN-γ in Group 2 was significantly higher compared to Group 1, the levels in both groups were lower than those in the Group 3 (p<0.05 and p<0.05, respectively). According to evaluation of neutrophil functions; no pathology was detected in Group 3, but the phagocytic activity and oxidative burst in Group 1 and Group 2 were lower compared to controls (p<0.001, p<0.01, respectively). In addition, both values were lolower in Group 1 compared to Group 2 (p<0,05).

**Conclusion:** Our results suggest that diabetic patients before insulin therapy, may have disturbances in neutrophil functions but with the improvement of metabolic control and less destruction in beta cells by decrease of the cytokines IFN-γ, IL-2 and especially TNF-α, neutrophil functions may improve.

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**Abnormalities in plasma 1,25-dihydroxyvitamin D3, and cellular 1-alpha hydroxylase and Cyclooxygenase-2 mRNA levels in recent-onset Type 1 diabetes**

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**Background and aims:** Epidemiological studies suggest that Vitamin D at birth protects individuals from type 1 diabetes perhaps by favouring maturation of the gut mucosa and by acting as an immune modulator of immune response. We investigated plasma levels of 1,25 dihydroxyvitamin D3 (1,25 (OH)2 D3) and peripheral blood mononuclear cells (PBMCs) mRNA levels of both 1-alpha hydroxylase (1-alpha OH), Vitamin D receptor (VDR) and Cyclooxygenase-2 (COX-2), a marker of inflammation in type 1 diabetes.
**Materials and methods:** PBMCs were isolated from 48 patients with recent-onset (<1 week) type 1 diabetes (22 males and 26 females, mean age 14.5 ± 5 years) and 27 age and sex-matched healthy subjects born and residing in the same region of continental Italy. Plasma levels of 1,25 (OH)2 D3 were measured using radio-immunoassay. Total RNA was analysed for 1-alpha OH, COX-2 and VDR mRNA levels using quantitative RT-PCR.
**Results:** Mean 1,25 (OH)2 D3 levels were lower in patients vs. controls (38 ± 29 vs 58 ± 29 pg/ml, $p<0.004$). There was no correlation between Vitamin D3 levels and patient demographics including metabolic status. We observed a compensatory increase in the enzyme that converts inactive to active 1,25 (OH)2 D3 in patients as shown by an increase in 1-alpha OH mRNA levels in patients compared to controls ($p=0.001$) while VDR mRNA levels remained normal. Interestingly COX-2 mRNA levels were significantly increased in patients vs. controls ($p<0.001$).
**Conclusion:** Our results indicate a deficiency in 1,25 (OH)2 D3 in recent-onset type 1 diabetes with a compensatory increase in 1-alpha OH mRNA levels. Increased COX-2 mRNA levels can be linked to either an inflammatory response or raised blood glucose levels at diagnosis. We conclude that Vitamin D may be an important pathogenic factor in type 1 diabetes and its administration as active form might be valuable therapy in limiting disease progression.

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**The inaugural phenotype of immune-mediated Type 1 diabetes varies according to age, gender and season at diagnosis but not HLA-DQ genotype**

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**Background and aims:** The incidence of type 1 diabetes varies according to age, HLA-DQ genotype, gender and season. We investigated whether these variables also influenced the clinical and biological characteristics in newly diagnosed patients since it is possible that the nature of the various combinations of external and genetic factors precipitating clinical onset may be (partly) reflected in the inaugural disease presentation.
**Materials and methods:** The inaugural disease phenotype and biological characteristics were assessed by questionnaire and central laboratory tests in 2176 antibody-positive diabetic patients aged 0–39 years diagnosed nationwide between 1989 and 2000 and compared after stratification according to age, HLA-DQ genotype, gender and season at diagnosis.
**Results:** Antibody-positive patients diagnosed under age 15 had more severe clinical presentation, lower random C-peptide levels, a higher prevalence of ICA, IAA, IA-2A and susceptible genotypes, and a lower prevalence of GADA and protective/neutral/rare genotypes, as compared to patients diagnosed after age 15 (all $p < 0.001$). Patients carrying the HLA DQ2/DQ8 genotype (n = 607) did not differ in clinical and biological characteristics from an equal number of age- and sex-matched subjects without this genetic accelerator except for a lower prevalence of the 5'INS I/I susceptibility genotype ($p < 0.001$) in the presence of DQ2/DQ8 above age 15. Compared to the entire group of 934 female patients, an equal number of individually age-matched male patients presented on average a shorter prodromal phase, higher glycemia, lower C-peptide levels and prevalence of GADA at diagnosis (all $p < 0.001$). The male excess in recent-onset type 1 diabetes was restricted to the high incidence season (high [November–February]: M/F = 1.46, low [June–September]: M/F = 1.02, $p = 0.001$). Patients were further compared according to season at diagnosis after matching for age and gender (n = 973 in each group; high season = October–March vs low season = April–September). C-peptide values were higher in the high incidence season as compared to the low incidence season ($p < 0.001$), especially in males.
**Conclusion:** The inaugural phenotype of immune-mediated type 1 diabetes varies according to age, gender and season at diagnosis but after adjustment for age and gender not to HLA-linked risk. Gender-and season-dependent etiological factors are likely to contribute to the observed gender- and season-dependent phenotypical heterogeneity.

# PS 32

# Genetics, diabetes and insulin resistance

## 515

**Genetic determination of metabolic syndrome base clinical components**

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**Background and aims:** Essential Hypertension (EH), abdominal obesity and Type 2 diabetes mellitus (DM) are the base clinical components of metabolic syndrome (MS). MS manifestation degree depends on presence of heredity predisposition to its components. The aim of the study was to assay the abdominal obesity and EH genetic determination and genetic correlation between EH, obesity and Type 2 DM.
**Materials and methods:** 240 probands with EH (BMI $28.127 \pm 0.76$ kg/m$^2$, WHR $0.87 \pm 0.06$), their 1st degree relatives (1249 subjects), 219 persons with abdominal obesity (BMI $35.33 \pm 0.98$ kg/m$^2$, WHR $0.98 \pm 0.04$), their 1st degree relatives (1174 ones) and 325 Type 2 DM patients (BMI $29.86 \pm 0.83$ kg/m$^2$, WHR $0.94 \pm 0.03$) and their 2008 1st degree relatives were enrolled. D.Falconer's and Ch.Smith's models were tested.
**Results:** The testing of the multifactorial threshold D.Falconer's model has been shown, that the heredity predisposition to EH and abdominal obesity correspond to parameters of this model. The hereditability coefficient "in narrow sense" (average means 68,0% for EH and 72,0% for obesity) obtained within the framework of the D. Falconer's model confirmed the important role of genetic factors in the appearance of this MS components. The decompose of phenotypic variances of the susceptibility, carried out on siblings and parents, has shown, that the most acceptable versions were decisions which included for EH estimation of additional component ($G_A$=67,60 ± 9,80%) and for abdominal obesity – both estimations of additional and dominant components – ($G_A$=71,36 ± 7,22% and $G_D$=4,56 ± 21,20%). Presence of genetic dominant component in the version of phenotypic variance decompose in abdominal obesity indicates on presence of nonlinear interallelic interactions in the determination of obesity. The study of MS components genetic heterogeneity within the framework of the S.Smith's model has shown the independence of gene complexes of predisposition to obesity, EH and Type 2 DM ($r_{gEH\text{-}DM}$=0,109; $r_{gEH\text{-}obesity}$=0,399; $r_{gDM\text{-}obesity}$=0,798).
**Conclusion:** It has been shown, that EH and obesity distribution in population and families may well be described by means of polygene model variants with significant influence of the major genes. Gene complexes of predisposition to obesity, EH and Type 2 DM are independent, however, their liabilities are covered.

## 516

**Prevalence of -132(G/A) mutation in the IAPP promoter gene in type 2 diabetic patients and clinical features of the carriers**

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**Background and Aims:** IAPP is the main component of the amyloid islet deposits found in most pancreas of type 2 diabetic subjects. Increased IAPP release could play a role in the pathogenesis of type 2 diabetes. Previous findings of our group demonstrated an increased transcription of the IAPP gene in subjects with the -132(G/A) mutation of the promoter region, and an increased prevalence of this mutation in type 2 diabetic subjects. The objectives of this study were to find out: 1 – The prevalence of the mutation in a population of type 2 diabetic subjects and in a non-diabetic population. 2 – The distinctive features of the subjects carrying the mutation, and 3 – The glucose tolerance state of the relatives of the carrier diabetic subjects.
**Patients and methods:** A total of 594 subjects (307 men, 287 women) were studied (356 with type 2 diabetes, 207 non-diabetic controls and 31 first-degree relatives of carrier diabetic subjects). The DNA obtained from peripheral blood was amplified by PCR. Abnormal bands found by SSCP were sequenciated to confirm the presence of the mutation. Different anthropometric, clinical and biochemical data were registered from all the diabetic subjects studied. A 75-gr, 2 h OGTT was performed to 14 carrier relatives, measuring glucose, insulin and IAPP concentrations. An adequate control group (n=18) without the mutation underwent the same OGTT in order to establish reference values.
**Results:** The mutation was found in 38 diabetic subjects (10.7%), 3 controls (1.4%) and 16 relatives (51.6%). When comparing diabetic subjects with and without the mutation, no differences were found related to age, sex, waist circunference, diabetes duration, treatment, diabetic complications or HbA1 c concentrations. However, the prevalence of hypertension was statistically significant in the diabetics carrying the mutation (74 vs 57%; $p<0.05$). In the relatives group we found 3 abnormal OGTT (1 with glucose intolerance and 2 diabetes). No differences were found in glucose and insulin values during the OGTT, but IAPP concentrations were higher (minutes 30 and 60) in the group of relatives compared with controls.
**Conclusions:** The prevalence of the -132 (G/A) mutation of the IAPP promoter region is higher in type 2 diabetic subjects and its relatives and it is associated with hypertension. The higher IAPP concentrations during OGTT in the relatives carrying the mutation could play a role in the development of type 2 diabetes in the future.
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**Study of the genetic background of the oGTT glucose curve types in Czech population**

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**Background and aims:** The oral glucose tolerance test (oGTT) is used to determine the status of glucose tolerance and insulin resistance. The aim of this study was to evaluate the genetic background of three types of glucose (Glc) curves defined by the cluster analysis of oGTT (Glc 0–180 min) derived data.
**Materials and methods:** The oGTT data from 296 biochemically and anthropometricly well characterized subjects (98 offspring of diabetic patients (O) and 198 controls (C) with non-diabetic parents) were analyzed. The common polymorphisms in INS VNTR (HphI), FABP2 (HpaI), PPARgamma2 (HgaI), B2AR (MvaI), B3AR (MvaI), UCP1 (BclI), Kir6.2 (BanII), ApoE (HhaI) genes were tested for the association with the type of the glycemic curve. The differences between the clusters defined by the K-means analysis were tested by Mann-Whitney test, the genotype frequencies were compared using chi-square test, Fisher exact test, resp. (NCSS 2001 statistical software, USA).
**Results:** Three distinct types of glycemic curves with signif. different $AUC_{Glc}$ were defined: (1) $AUC_{Glc}$ = 1146 ± 102 (n=96; sex 57 M/39 F; mean age 37 ± 12 yr; 43.8% O; 93 normal Glc tolerance NGT/ 3 impaired Glc tolerance IGT), (2) $AUC_{Glc}$ = 876 ± 91 (n=181; 54 M/127 F; 31 ± 10 yr; 26.0% O; 181 NGT/ 0 IGT), (3) $AUC_{Glc}$ = 1443 ± 192 (n=19; 6 M/13 F; 41 ± 12 yr; 47.4% O; 7 NGT/ 12 IGT). These clusters signif. differed in age ($p_{12}$=0.00004; $p_{23}$=0.0005); insulin ($AUC_{IRI}$: $p_{12}$=0.000005, $p_{23}$=0.00002; $p_{13}$=0.009); HOMA F ($p_{12}$=0.016); HOMA R ($p_{12}$=0.00005, $p_{23}$=0.01); disposition indices, lipid spectra, serum uric acid, growth hormone etc. The anthropometric parameters were evaluated separately for men and women after the adjustment for age. In men the signif. dif. in BMI, waist circumference, WHR and muscle/fat mass % were detected whereas the women differed only in the last parameter. Among tested genetic variants the association of PPARgamma2 (Pro12Ala) genotype with glycemic curve type was found (AA/AP/PP: (1) 0%/34,6%/65.4%, (2) 2.2%/19.9%/77.9%; (3) 7.7%/7.7%/84.6%, Fisher exact test, $p_{12}$=0.029, $p_{13}$=0.0099).
**Conclusion:** Cluster analysis revealed three distinct types of oGTT derived glycemic curves determined in offspring of diabetic patients and healthy controls. These clusters signif. differed in many metabolic and anthropometric parameters. Among many genetic and environmental components underlying the glucose tolerance status, the PPARgamma2 (Pro12Ala) polymorphism appears to be associated with the type of glycemic curve.
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**The common PPAR-γ2 Pro12Ala variant is associated with greater insulin sensitivity**

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**Background and aims:** Peroxisome Proliferator-Activated Receptor-γ2 (PPAR-γ2) is a nuclear receptor involved in transcription of target genes. Several genetic variants have been identified; Pro12Ala is a missense mutation in exon 2 of the PPAR-γ2 and it's highly prevalent in Caucasian populations. Conflicting conclusions about the association between this mutation and complex traits, such as obesity, insulin sensitivity and type 2 diabetes (T2DM) have been reported.
**Material and methods:** We have investigated the role of PPAR-γ2 Pro12Ala polymorphism in the insulin sensitivity in two different populations: a) obese children (n=200; mean age 10.38 ± 2.8; BMI-SDS 2.78 ± 0.71) and b) adult subjects (n=1215; mean age 42.6 ± 13.6; BMI=32 ± 9) in whom clinical and biochemical analyses were performed. Two hundred bp of sequence surrounding PPAR-γ2 Pro12Ala was provided to Applied Biosystems to develop Taqman Allelic Discrimination Assays using their assay by design platform (ABI, Foster City, CA). Genotyping of the Pro12Ala was performed by optical seals on a 7900HT plate reader; individual genotypes were determined using SDSv2.1 software (ABI, Foster City, CA).
To estimate the insulin sensitivity status the homeostasis model assessment of insulin resistance (HOMA-IR) was calculated in all subjects. Oral Glucose Tolerance Test (OGTT) was also performed and the Matsuda Insulin Sensitivity index (ISI) calculated in obese/overweight adults (BMI >25). The effect of the Pro12Ala polymorphism on the quantitative variables was investigated using multiple linear regression analysis.
**Results:** The frequency of Ala allele was 9%, similar to that reported in other Caucasian populations. The X12Ala (either Pro12Ala or Ala12Ala) genotype was associated with significantly lower fasting insulin levels compared to Pro/Pro in children (p=0.01) and in adults (p=0.001). Consistent with this finding, significant lower HOMA-IR was observed in X12Ala carriers (p=0.03 in children; p=0.002 in adults). Moreover, among the overweight/obese subjects Ala carriers showed a significantly higher ISI index than wild type individuals (p=0.01).
**Conclusion:** In conclusion, our observations demonstrate that the X12Ala variant is significantly associated with greater insulin sensitivity in both populations. X12Ala carriers may be protected from insulin resistance syndrome by the strong phenotypic effect of the Ala 12 allele of PPAR-γ 2 gene.
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**Exogenous insulin decreases gene expression in bone but not in liver and spleen of spontaneously diabetic BB/OK rats**

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**Background and aims:** It is well known that type 1 diabetes is associated with a decrease in bone mass and delayed healing of fractures in humans and in animal models, which is largely explained by diminished or affected gene expression. Using well- and poorly-compensated diabetic BB rats spontaneously developing insulin-dependent type 1 diabetes, it was clearly shown that the metabolic state of rats markedly influenced the extent of delayed bone healing in the early period. To establish mechanisms to explain deficient fracture healing in poorly compensated BB rats, we studied the relative expression of genes in bone involved in bone repair (*Bmp-1, Bmp-4, Bglap, Vegf, Il-1b, Tgfb1, Yy1, Sp1*) compared with liver and spleen.
**Materials and methods:** Six age-matched non-diabetic BB males were compared with 12 diabetic BB males which were either well compensated (n=6) by implanting osmotic insulin pumps or were poorly compensated (n=6) by one daily application of 1 IU insulin. After 4 weeks of insulin treatment, animals were killed and liver, spleen, and tibial bone, excluding all cartilaginous and soft tissue, were removed. Total RNA of bone, liver and spleen was extracted, transcribed into complementary DNA, and used for real-time PCR (ABIPrism7000).
**Results:** There were no significant differences between well- and poorly-compensated rats in terms of age, blood glucose values, and body weight at diabetes onset. Four weeks later, the metabolic state was significantly different between well- and poorly-compensated rats. Diabetic rats compared to non-diabetic rats showed expression that was generally reduced by 50% or more in bone, but not in liver or spleen. In bone, significant differences were found between non-diabetic and both well- and poorly-compensated diabetic animals in terms of the relative gene expression of *Bmp-4, Bglap, Vegf, Yy1* and *Sp1*. Although similar differences were also observed in *Bmp-1* and *Il-1b*, significant differences were only found between non-diabetic and poorly-compensated rats. No differences between non-diabetics and well- or poorly-compensated diabetics were observed in relative expression of *Tgfb1*.
**Conclusions:** In this study, we provide a first evidence that diabetes in general and insulin in particular affect bone, but not liver and spleen gene expression in spontaneously diabetic BB rats, which may be responsible for the delayed bone defect healing in the poorly-compensated diabetic state.

## 520

**High frequency of exon-3 deleted polymorphism of the growth hormone receptor gene (GHRd3) in Type 1 diabetics from Chile**

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**Background and aims:** Type 1 diabetes is a multifactorial autoimmune disease characterized by the destruction of β cells with both environmental (virus or diet) and genetic factors contributing to the development of the disease. Both, wild type (GHRwt) and the exon 3 deletion isoform (GHRd3) has been identified and both are expressed in liver, pancreas, stomach and small intestine. A high expression of mRNA of GHR gene in mucosal gut suggest the possible role on digestive and immune functions. To date the functional importance of the GHR domain encoded by exon 3 is unknown.We investigate the putative effect of the allelic variant GHRd3 on cytokine profile in type 1 diabetic children.
**Materials and methods:** A control population (n=96, age: 12.2 ± 4.5 years) and recent diagnosis type 1 diabetic children (n=111, age: 8.7 ± 3.6 years) were analyzed for height, birthweight, IL-1β, IL-2, IL-4, TGFβ1 and INFγ cytokines profile. GHRd3 polymorphism was determined by means of multiplex PCR.
**Results:** The allele frequency for d3 allele was 32.8% in type 1 diabetics and 25.1% in controls. No statistical significant difference was observed in Type 1 diabetics or controls regarding to GHRd3 polymorphism and height or birthweigh. Among type 1 diabetic children, the d3 polymorphism was associated with a significant high levels of IL-1β ($p<0.05$).

Table 1: GHRd3 carriers and cytokine level in type 1 diabetes (mean value and range)

| GHRd3 | IL-1β (pg/ml) | IL-2 (pg/ml) | IL-4 (pg/ml) | TGFβ1 (ng/ml) | INFγ (pg/ml) |
|---|---|---|---|---|---|
| f1/f1 | 8.9 | 20.8 | 9.8 | 1.2 | 22.4 |
| (n=68) | (2.7–192.2) | (3.2–90.3) | (7.2–32.6) | (0.3–4.2) | (7.6–50.8) |
| d3 carriers | 15.5* | 17.5 | 8.9 | 1.4 | 21.3 |
| (n=43) | (5.0–87.0) | (3.0–59.8) | (7.0–18.5) | (0.5–2.8) | (8.7–33.4) |

*$p<0.05$

**Conclusion:** A high frequency of d3 isoform was found in Chilean population. In type 1 diabetic children, the carries of d3 isoform shown higher levels of IL-1β compared with non carriers. The regulatory importance of this isoform on expression of proinflammatory cytokines need to be elucidated.
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## 521

**Aromatase gene (CYP19) haplotype and insulin resistance are independently associated with polycystic ovary syndrome risk in both girls and young women**

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**Background and aims:** Aromatase (CYP19) catalyses the conversion of androgens to oestrogens. Rare loss of function mutations in CYP19 result in severe androgen excess. We hypothesised that common genetic variation in the CYP19 gene contributes to androgenaemia in healthy women, and also confers risk for hyperandrogenic, insulin resistant conditions such as premature pubarche (PP) and Polycystic Ovary Syndrome (PCOS).

**Materials and Methods:** We genotyped DNA at 4 haplotype tag single nucleotide polymorphisms (htSNPs) in the coding region of the CYP19 gene: 1) in healthy young female volunteers from Oxford, UK (mean age 22 yr, n=109), who were not on oral contraception; 2) in PP girls (mean age 11 yr, n=181) and control girls (mean age 11 yr, n=144) from Barcelona, Spain. Insulin sensitivity was assessed from fasting glucose and insulin concentrations (calculated as HomaS). CYP19 haplotypes were reconstructed, and clinical and biochemical features of PCOS were compared by haplotype or genotype.

**Results:**

Cohort 1) In healthy young women, an intron 2 A/G SNP was associated with higher plasma testosterone levels (p=0.02), and with a higher number of PCOS features (from: menstrual irregularity; acne; hirsuitism; testosterone >3 nmol/l; and LH>10 IU/l) (p=0.008, Table). In a covariate model, number of PCOS features was independently associated with both CYP19 genotype (p<0.05) and lower insulin sensitivity (p=0.006).

Aromatase (CYP19) intron 2 A/G SNP genotype

| | A/A (n=20) | A/G (n=61) | G/G (n=28) | Additive model | Dominant model |
|---|---|---|---|---|---|
| Number of PCOS features (mean, SD) | 1.7 (0.8) | 1.7 (1.0) | 1.2 (0.9) | p=0.03 | p=0.008 |
| Plasma testosterone (nmol/l) (geometric mean, 95% CI) | 2.6 (2.3~3.0) | 2.5 (2.3~2.7) | 2.2 (2.0~2.4) | p=0.05 | p=0.02 |

Cohort 2) In PP girls the AAGG haplotype was more prevalent than in control girls (16% vs 9%, p<0.01). In contrast the AGGG haplotype was more common in controls than in PP girls (26% v. 18%, p<0.05). Relative to the AGGG haplotype, therefore, the odds ratio for having PP associated with the AAGG haplotype was 2.5 (1.4~4.4). These differences could mainly be explained by differences in genotype frequency of the same intron 2 A/G SNP (PP vs Controls: A/A: 44% vs 26%; A/G: 32% vs 48%; G/G: 25% vs 26%, p=0.001).

Among 84 post-pubertal girls from both cases and controls, risk of ovarian hyperandrogenism was independently associated with both CYP19 haplotype (p<0.05) and lower insulin sensitivity (p<0.05).

**Conclusion**: These findings, in two independent cohorts, show that insulin resistance and common genetic variation in the CYP19 gene are independently associated with androgenaemia and PCOS risk in both girls and young women.

## 522

**Cloning and functional characterisation of porcine melanin-concentrating hormone receptor 1 (MCH1-R)**

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**Background and aims:** The neuropeptide melanin-concentrating hormone (MCH) is involved in the regulation of feeding as well as fluid and energy homeostasis. MCH mediates its effects through activation of G-protein coupled receptors (GPCR), two of which – termed MCH1-R and MCH2-R – have recently been cloned from human RNA sources. Antagonists of the MCH1-R are potent anorexic agents with promising weight-loss properties and may thus provide a novel therapeutic principle to treat obesity and associated disorders.

The MCH2-R is absent or non-functional in rodents. For pharmacological profiling of MCH1-R antagonists with potential cross-reactivity to MCH2-R higher species like the pig (*Sus scrofa*), which possesses the MCH2-R, will be of importance. Furthermore, pigs have been shown to be a useful animal model of obesity and the metabolic syndrome.

In order to better understand the effects of MCH and/or MCH-R antagonists in this animal, we have cloned and characterised porcine MCH1-R.

**Materials and methods:** A full length pig MCH1-R cDNA was obtained by a PCR-based approach using total RNA isolated from porcine cerebral cortex. After sub-cloning into the vector pcDNA3.1/Zeo, the DNA was subjected to double-stranded sequencing. A CHO-$G_{alpha}16$ cell line was stably transfected with the DNA construct. The recombinant pig MCH1-R was then characterised in receptor binding studies using $^{125}$I-human MCH as radioligand. Inhibition of cyclic AMP accumulation (induced by $10^{-5}$M forskolin) was measured using a Flashplate Assay (Amersham). Finally, utilizing the $G_{alpha}16$ signalling properties, the recombinant pig MCH1-R was also examined in a FLIPR assay (Molecular Devices), measuring intracellular $Ca^{2+}$-mobilisation.

**Results:** DNA sequencing of the cDNA encoding the putative porcine MCH1-R revealed 97%, 97%, and 94% protein sequence homology to human, rhesus or rat MCH1-R, respectively. Sequence homology was highest in the proposed transmembrane regions of the predicted GPCR, with highest sequence variability between species in the proposed extracellular amino terminus of the receptor protein. Expression of the receptor protein at the cell surface was verified by receptor ligand bindig studies. The peptide human MCH displaced the radioligand $^{125}$I-MCH with a pKi value of 9.25 +/- 0.008; pIC50 9.03 +/- 0.24 (n=2). Furthermore, MCH1-R was reported to be negatively coupled to adenylate cyclase, via Gi/Go, thus stimulation of this receptor leads to inhibition of cyclic AMP levels. In line with previous findings on human MCH1-R, forskolin ($10^{-5}$M) induced cyclic AMP formation was inhibited by MCH with an pIC50 value of 8.37 +/–0.41 SEM (n=3). In further functional experiments using FLIPR technology, human MCH, Phe$^{13}$-Tyr$^{19}$-MCH, and salmon MCH induced $Ca^{2+}$-mobilisation with pEC50 values of 8.8, 8.7 and 8.6, respectively. All compounds showed full agonism in this assay. These data are well comparable to recombinant human MCH1-R (pEC50 MCH: 8.3).

**Conclusion:** In summary, we have cloned and characterised the pig MCH1-R. The recombinant receptor protein showed a high degree of homology to human and rodent MCH1-R with very similar pharmacological properties in vitro. This provides the basis for the potential utilization of domestic pigs as in vivo models to study the effects of MCH1-R antagonists.

# PS 33
# Myotubes and skeletal muscle

## 523

**Nutrient-dependent regulation of interleukin-6 (IL-6) expression and metabolic effects of IL-6 in human myotubes**

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**Background and aims:** Circulating interleukin 6 (IL-6), insulin and also free fatty acids (FFA) concentrations are associated with impaired insulin action in obese and type 2 diabetic individuals. Skeletal muscle represents a major target of impaired insulin action, however, a causal relationship between elevated plasma FFA, IL-6 and insulin resistence in skeletal muscle cells has not been shown. Therefore, we studied whether FFA may affect IL-6 expression in human myotubes and we investigated the possible effects of IL-6 on metabolic pathways in myotubes.
**Materials and methods:** Human myotubes were obtained from muscle biopsies. Cells were treated with 0.25 mM saturated or unsaturated fatty acid for 20 h unless indicated otherwise (BSA was used as control), or 30 mM glucose and insulin for 48 h, or 5 nM TNF-a. IL-6 mRNA expression was measured by realtime PCR; IL-6 protein in the supernatant by ELISA; activation of NF-kB and Ik-B proteins was detected by EMSA and Western blotting. Inhibitors of proteasome activity (MG 132), oxidative stress (a-lipoic acid), acyl-CoA synthetase (triacsin C) and ceramide synthesis were used as indicated. To study the effects of IL-6 on myotubes, cells were co-stimulated with IL-6 alone or costimulated with IL-6 and insulin. Metabolic pathways were studied by Western blotting; mRNA expression of insulin signaling molecules was determined by realtime PCR and glycogen synthesis was measured using 14C-glucose.
**Results:** We demonstrate that specifically saturated FFA, e.g. palmitate (0.25 mM), induce IL-6 mRNA expression and protein secretion, by a proteasome-dependent mechanism, that leads to a rapid and chronic activation of NF-kB. Insulin, high glucose concentrations or unsaturated FFA did not activate IL-6 expression. In fact, the unsaturated FFA linoleate inhibited palmitate-induced IL-6 production. Since inhibition of palmitate metabolism by the acyl-CoA synthetase inhibitor triacsin C did not abolish IL-6 expression, it appears that the palmitate molecule per se exerts the observed effects. Furthermore, we show that in human myotubes IL-6 activates the phosphorylation of signal transducer and activator of transcription (STAT-3) in concentrations similar to hepatocytes. However, no inhibitory effect of IL-6 on insulin action, determined as phosphatidylinositol 3-kinase (PI 3-kinase) association with insulin receptor substrate-1 (IRS-1), Akt phosphorylation and glycogen synthesis, was detected. Moreover, IL-6 treatment of myotubes induce the phosphorylation of Akt on serine-473, and no reduction of IRS-1 or PI-3 kinase p85 subunit mRNA expression was observed.
**Conclusion:** We conclude that IL-6 expression may be modulated by the composition of circulating FFA e.g. by diet. Myotubes are target cells for IL-6 with a sensitivity similar to hepatocytes and adipocytes. In contrast to these cell types, no IL-6-mediated reduction of insulin-dependent pathways was detected in myotubes.
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## 524

**Myotubes established from Type 2 diabetic subjects showed no expression of an increased long-chain acyl-CoA level**

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**Background and aims:** Accumulation of intramuscular long-chain acyl-CoA esters has in animal and human models previously been suggested to play an important role in lipid-induced insulin resistance. The aim of this study was to examine whether myotubes established from lean controls and subjects with type 2 diabetes (T2D) express differences in the intracellular long-chain acyl-CoA ester pool when cultured without exogenous fatty acids.
**Materials and methods:** Human myotubes established from control subjects and T2D subjects were allowed to differentiate for eight days without supplementation of exogenous fatty acids. Acyl-CoA were extracted, converted to fluorescent etheno acyl-CoA by derivation and detected by HPLC.
**Results:** In myotubes cultured without supplementation of exogenous fatty acids (basal state), we were unable to detect any significant difference in the size and composition of the long-chain acyl-CoA ester pool between lean controls and diabetics (Tabel 1). Data are presented as mean±SE. Total pool of long-chain acyl-CoA esters was for non-diabetics 109,04 pmol/mg protein ± 3,97 and for diabetics 106 pmol/mg protein ± 6,98. For the palmiotyl-CoA fraction, the results were 19,60 pmol/mg protein ± 0,81 for the non-diabetic compared to those of the diabetics: 19,48 pmol/mg protein ± 1,29. For the oleic-CoA fraction, the same was found: 52,11 pmol/mg protein ± 2,02 for non-diabetics and 51,36 pmol/mg protein ± 3,42 for diabetics.
**Conclusions:** No differences were shown in the acyl-CoA esters composition or in the total pool of acyl-CoA esters between myotubes established from control and T2D. Even though we were not able to show an accumulation of acyl-CoA in diabetic myotubes when cultured without exogenous fatty acids, long-chain acyl-CoA levels may be different under exposure to exogenous fatty acids.

Table 1

| Acyl-CoA | Control | SE | Type 2 diabetes | SE |
|---|---|---|---|---|
| C14 | 3,78 | 0,75 | 3,67 | 0,67 |
| C16 | 19,6 | 0,81 | 19,48 | 1,29 |
| C16:1 | 2,89 | 0,32 | 2,37 | 0,40 |
| C18 | 2,48 | 0,23 | 1,84 | 0,36 |
| C18:1 | 52,11 | 2,02 | 51,36 | 3,42 |
| C18:2 | 16,41 | 0,93 | 16,26 | 1,52 |
| C18:3 | 0,88 | 0,11 | 1,01 | 0,10 |
| C20:4 | 10,89 | 0,56 | 10,52 | 0,67 |
| Total | 109,04 | 3,97 | 106,52 | 6,98 |

## 525

**Stearoyl-CoA desaturase impairs insulin signaling and glucose uptake in skeletal muscle**

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**Background and aims:** Insulin resistance in skeletal muscle is a hallmark of type 2 diabetes. We previously reported differentially regulated genes in skeletal muscle of young insulin resistant Zucker diabetic fatty (ZDF) rats and found a strongly increased expression of stearoyl-CoA desaturase (SCD isoform 1) that correlated with highly elevated amounts of palmitoleoyl-CoA (16:1) fatty acid species in this tissue. Here, we describe the generation of a cellular model of human SCD in skeletal muscle and analyze the effects of SCD expression on insulin signaling and fatty acid metabolism.
**Materials and methods:** SCD overexpressing L6GLUT4 myc myoblasts (SCD cells) were generated and characterized by western blotting, immunofluorescence and by monitoring lipid accumulation. We compared these SCD cells to control cells regarding insulin-stimulated glucose uptake, insulin signaling, protein and transcript expression levels of regulators of insulin action and differential effects of high glucose and fatty acid treatment on glucose uptake and fatty acid metabolism.
**Results:** SCD cells were functional regarding SCD expression, subcellular localization and increased accumulation of neutral lipids upon palmitate treatment. Compared to controls, SCD cells revealed impaired basal and insulin-stimulated glucose uptake in 2-deoxyglucose uptake assays (average: 2.3-fold decreased). Furthermore, SCD cells exhibited decreased tyrosine phosphorylation of IRS-1 (decreased by 1.8-fold basal and 12.9-fold insulin stimulated) and consequently decreased downstream AKT-phosphorylation (decreased by 1.3-fold basal and 3.5 fold insulin stimulated). Impaired insulin-signaling and glucose disposal might be caused by effects of SCD on gene and protein expression: in SCD cells elevated amounts of PTP-1B protein (1.45-fold over controls) and decreased GLUT4 transcript levels (1.7-fold below controls) were detected. Upon treatment with high glucose and high fatty acid conditions, no further impairment in insulin sensitivity could be observed for SCD cells, whereas control cells became insulin resistant (maximum insulin stimulation reduced to 58%). These findings could be correlated with changes in the long-chain acyl-CoA profile of SCD and WT cells.
**Conclusion:** Various steps of the insulin signaling cascade are impaired by elevated amounts of SCD, resulting in impaired glucose uptake. Our observations point towards the existence of both, direct effects of SCD or the products of its enzymatic activity on immediate signaling events as well as indirect downstream effects on the level of (post-) transcriptional regulation.

## 526

**Selective inhibition of glycogen synthase kinase-3 upregulates insulin signaling in isolated insulin-resistant rat skeletal muscle**

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**Background and aims:** Glycogen synthase kinase-3 (GSK3), a serine/threonine kinase, has been implicated in the multifactorial etiology of skeletal muscle insulin resistance in animal models and in type 2 diabetic human subjects. However, limited information is available on the potential molecular mechanisms of GSK3-associated insulin resistance in mammalian skeletal muscle. In the present study, we determined if selective GSK3 inhibition in vitro leads to an improvement in insulin action on glucose transport activity in isolated skeletal muscle of insulin-resistant, prediabetic obese Zucker (fa/fa) rats, and if these effects of GSK3 inhibition are associated with enhanced insulin signaling.
**Materials and methods:** Soleus (mainly type I fibers) and epitrochlearis (predominantly type IIb fibers) from female obese Zucker rats were incubated in the absence or presence of a selective, small organic GSK3 inhibitor (1 μM CT118637, $K_i$<10 nM for GSK3α and GSK3β). Maximal insulin stimulation (5 mU/ml) of glucose transport activity (assessed by 2-deoxyglucose uptake), glycogen synthase (activity ratio −/+ G6P), and select insulin signaling factors (tyrosine phosphorylation of insulin receptor and IRS-1 and serine phosphorylation Akt and GSK3α/β) were then assessed.
**Results:** Selective GSK3 inhibition in vitro enhanced ($p<0.05$) basal glycogen synthase activity and insulin-stimulated glucose transport activity in obese type IIb epitrochlearis (81% and 24%) and obese type I soleus (108% and 20%) muscles. GSK3 inhibition did not modify insulin-stimulated tyrosine phosphorylation of insulin receptor in either insulin-resistant muscle type. However, in obese soleus muscles, selective GSK3 inhibition enhanced insulin-stimulated tyrosine phosphorylation of IRS-1 by 45%, serine phosphorylation of Akt by 30%, and serine phosphorylation of GSK3 by 39%. Substantially smaller GSK3 inhibitor-mediated enhancements of insulin action on these insulin signaling factors were observed in obese epitrochlearis muscles (13–15%).
**Conclusion:** The results of this study indicate that selective GSK3 inhibition enhances insulin action in insulin-resistant skeletal muscle of the pre-diabetic obese Zucker rat, at least in part by relieving the deleterious effects of GSK3 action on post-insulin receptor insulin signaling, including IRS-1, Akt, and GSK3. These effects of GSK3 inhibition on insulin action are greater in type I muscle than in type IIb muscle from these insulin-resistant animals.
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## 527

**Direct effects of an agonist of PPARdelta on fuel metabolism of isolated rat skeletal muscle**

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**Background and aims:** Agonists of peroxisome proliferator-activated receptors (PPAR) gamma and alpha are used for the treatment of type 2 diabetes and lipid disorders, and their regulatory roles in fuel metabolism have been thoroughly studied. The third subtype, PPARdelta, has so far attracted much less interest. Recently, however, it has been reported that administration of GW501516, a specific PPARdelta agonist, ameliorated insulin resistance in obese rodents, which suggests that PPARdelta could also be a target for antidiabetic treatment. Based on the observation that PPARdelta is abundant in skeletal muscle, where it mediates increased cellular fatty acid utilisation, the present study examines, if and in what manner the PPARdelta agonist GW501516 directly affects glucose metabolism of rat skeletal muscle.
**Materials and methods:** Freshly isolated soleus muscle strips obtained from male Sprague-Dawley rats were exposed to various concentrations of GW501516 *in vitro*. During the last hour of the incubation, rates of fuel metabolism were measured under stimulation with 100 nmol/l insulin.
**Results:** In line with other reports, 24 h of exposure to GW501516 increased palmitate oxidation in isolated muscle specimens (all data given as % change *vs* an intraindividual control muscle: 10, 100, and 1000 nmol/l GW501516: +20 ± 15%, ns; +49 ± 16%, $p<0.02$; and +46 ± 10%, $p<0.001$). In parallel, GW501516 dose-dependently decreased glucose utilization as indicated by reduced rates of aerobic and anaerobic glycolysis (glucose oxidation: −11 ± 3%, $p<0.01$; −31 ± 7%, $p<0.002$; and −46 ± 8%, $p<0.001$; lactate release: −4 ± 2%, ns; −13 ± 3%, $p<0.001$, and −20 ± 2%, $p<0.001$), and by reduced glycogen storage (rate of glucose incorporation into glycogen: −6 ± 5%, ns; −31 ± 4%, $p<0.001$; and −42 ± 6%, $p<0.001$; glycogen content at the end of the experiment: +8 ± 10%, ns; −38 ± 8%, $p<0.001$; and −24 ± 8%, $p<0.01$). Protein synthesis was not affected (rate of methionine incorporation into protein: +1 ± 4%; +6 ± 12%; and +2 ± 7%; all ns). In line with a presumptive genomic mode of action via activation of PPARdelta, the effects of GW501516 on glucose metabolism were delayed (no effects of 1 umol/l GW501516 within 1.5 and 5 h: glucose oxidation, +13 ± 6% and −8 ± 5%, respectively; both ns) and were abolished by an inhibitor of protein synthesis (% change in glucose oxidation by 1 umol/l GW501516 in the absence vs presence of 1 g/l cycloheximide: −33 ± 6% *vs* +11 ± 8%, $p<0.002$). Of note, the decrease in glucose utilisation persisted, when the GW501516-induced increase in fatty acid oxidation was counteracted by concomitant exposure to etomoxir, a specific inhibitor of fatty acid oxidation (% change induced by 1 umol/l GW501516 in the absence *vs* presence of 10 umol/l etomoxir: palmitate oxidation: +49 ± 11% *vs* −5 ± 10%, $p<0.005$; glucose oxidation: −32 ± 5% *vs* −29 ± 5%, ns).
**Conclusion:** The findings suggest that activation of PPARdelta by GW501516 in skeletal muscle reduces glucose utilisation, which is not a direct and immediate result of the concomitant increase in fatty acid oxidation. The described effects of GW501516 on fuel handling of skeletal muscle could be causal for its reported antidiabetic action.

## 528

**Investigations of Glucotransporter 4 (GLUT4) expression in lymphocytes of healthy subjects and Type 2 diabetic patients**

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**Background and aims:** Various metabolic conditions influence lymphocyte function. This relationship can be particularly expressed by glucose transporter proteins in lymphocytes. The earlier obtained results revealed significant differences in the deoxy-D-glucose uptake in Type 2 diabetic patients. These differences did not originate from the alteration of expression of GLUT1 and GLUT3 proteins. The aim of this study was to determine the presence of insulin-dependent GLUT4 in human lymphocytes, which has not been proven so far, and to evaluate if there was a change of GLUT4 expression in lymphocytes obtained from diabetics in comparison with healthy subjects.
**Materials and methods:** The study group included 15 Type 2 diabetic patients treated with diet only. As a control group 15 matched healthy subjects were enrolled. The expression of GLUT4 was investigated by immunocytochemical method and flow cytometry. Circulating lymphocytes from human peripheral blood were obtained from heparinised blood by Ficoll-Isopaque gradient centifugation. Indirect immunofluorescence was applied as a staining technique. Cells were stained by using anti-human GLUT4 antibody and anty-IgG2-FITC as an isotype control. In the case of immunocytochemistry, as a second antibody, anti-rabbit IgG labelled with peroxidase was used. Flow cytometry was performed utilizing a FACSCalibur (Becton-Dickinson). The data was analyzed using Cell Quest software and presented as a percentage of lymphocytes revealing expression of the determined receptor protein.
**Results:** An immunocytochemical method showed the presence of GLUT4 proteins in lymphocytes obtained from Type 2 diabetic patients and healthy subjects. Much more intensive reaction was observed in diabetic patients. In the case of flow cytometry, the expression of GLUT4 in Type 2 diabetic patients was found in 24% (± 2%) of the total count of lymphocytes, whereas in healthy subjects this number was only 12% (± 1,5%). This difference is very significant.
**Conclusion:** The presence of GLUT4 in lymphocytes of healthy subjects and Type 2 diabetic patients has been proved. This phenomenon has not been revealed so far. The expression of GLUT4 in diabetic patients was twofold higher in comparison with healthy subjects. It shows the adaptation of lymphocytes to incorrect metabolic conditions (hyperglycemia).

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**Divergent roles of Epac and protein kinase a on signalling pathways downstream of protein kinase-b in skeletal muscles: implications for cross talk between insulin and adrenaline**

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**Background and aims:** Skeletal muscle is the major tissue for insulin-stimulated glucose uptake and therefore pivotal for regulation of blood glucose concentration. Adrenaline and insulin are the two most important hormones regulating glucose metabolism in muscle with insulin's effects generally requiring PKB and adrenalines effects requiring cAMP and PKA. Recent evidence indicates cAMP can regulate PKB in some cell types via Epac (Exchange protein directly activated by cAMP). Epac activates the small GTP-binding protein Rap1. This suggests possible crossover between insulin ad adrenaline signalling but it is not clear if this happens in muscle. These interactions may be particular interesting to understand since adrenaline completely blocks insulin-stimulated glycogen synthesis but adrenaline does not inhibit insulin-stimulated glucose transport.
**Materials and methods:** Rat soleus muscles were incubated in Krebs-Henseleit buffer with insulin (10 mU/ml) and adrenaline ($10^{-6}$ M). Phosphorylation of proteins was measured with phosphospecific antibodies. Kinase activities were measured after immunoprecipitation with appropriate substrate.
**Results:** Adrenaline alone did not influence PKB activation. Surprisingly, adrenaline dramatically potentiated insulin-stimulated phosphorylation of PKB (both $Ser^{473}$ and $Thr^{389}$). Adrenaline also potentiated PKBα and PKBβ enzyme activities. These effects were inhibited by wortmannin but adrenaline did not increase insulin-stimulated p85α PI 3-kinase activity. Adrenalines potentiated insulin-stimulated PKB activation via β-adrenergic receptors and accumulation of cAMP. However, the PKA inhibitor H89 did not block the potentiating effect of adrenaline on insulin-stimulated PKB activation. Instead, blockade of PKA by H89 further increased insulin-stimulated PKB activation, which suggests that PKA inhibits PKB activation. On the other hand, the Epac activator 8-(4-chlorophenylthio)-2`-O-methyl-cAMP potentiated insulin-stimulated PKB phosphorylation as adrenaline did. Further, while adrenaline and Epac activation alone did not promote $p70^{S6K}$ $Thr^{389}$ phosphorylation they potentiated insulin effects. In contrast adrenaline alone stimulated GSK-3 phosphorylation and increased insulin-stimulated GSK-3 phosphorylation. Epac activation did not increase GSK-3 phosphorylation alone. Surprisingly, despite Epac activation increased insulin-stimulated PKB phosphorylation, Epac activation did not increase insulin-stimulated GSK-3 phosphorylation.
**Conclusion:** The studies provide evidence that adrenaline, via cAMP and Epac, potentiates the effects of insulin on PKB activation. The results indicate that Epac acts on a particular pool of PKB that allows insulin and β-adrenergic stimulation to amplify effects on certain cellular responses such as those involved in protein synthesis.

# PS 34

# Adipocyte/insulin sensitivity cross talk

## 530

**TNF-α gene G-308A polymorphism and body fatness in the HERITAGE Family Study**

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**Background and aims:** The tumor necrosis factor alpha (TNF-α) gene promoter polymorphism G-308A has been associated with obesity, but findings have not been consistent. We investigated the association between this TNF-α gene polymorphism and measures of body fat in non-diabetic white and black men and women.
**Materials and methods:** Body mass index (kg/m²), fat mass (underwater weighing), and abdominal total, subcutaneous and visceral fat areas (computed tomography) were measured in 493 White (243 men, 250 women) and 236 Black (87 men and 149 women) subjects aged 17–65 years in the HERITAGE Family Study. The TNF-α G-308A polymorphism was determined using polymerase chain reaction amplification followed by digestion with restriction enzyme NcoI. Data were analysed using Chi-square test and the MIXED procedure of SAS.
**Results:** Genotype frequencies for AA, AG and GG were 2.4% (n=12), 26.0% (n=128) and 71.6% (n=353) in Whites and 3.4% (n=8), 21.6% (n=51) and 75.0% (n=177) in Blacks, respectively, and they were in Hardy-Weinberg equilibrium. Because AA homozygotes differed from G allele carriers, AG and GG genotypes were combined in subsequent analyses. The associations between the G-308A polymorphism and measures of body fat in Whites and Blacks after adjustment for age and sex are presented in the table. The percentage differences between AA and AG/GG groups are also shown.
**Conclusion:** These data suggest that the TNF-α gene G-308A polymorphism contributes to variability in body fatness.

| | | Whites (n=493) Mean (SEM) | Difference between groups (%) | Blacks (n=236) Mean (SEM) | Difference between groups (%) |
|---|---|---|---|---|---|
| Body mass index (kg/m²) | AA | 30.7 (1.6) | | 30.7 (2.3) | |
| | AG/GG | 25.8 (0.2) | 19.0 | 28.3 (0.5) | 8.5 |
| | p | 0.003 | | 0.312 | |
| Fat mass (kg) | AA | 27.1 (3.6) | | 32.5 (4.8) | |
| | AG/GG | 20.4 (0.5) | 32.8 | 25.2 (1.1) | 29.0 |
| | p | 0.069 | | 0.149 | |
| Abdominal total fat (cm²) | AA | 483 (57) | | 511 (82) | |
| | AG/GG | 350 (9) | 38.0 | 374 (18) | 36.6 |
| | p | 0.022 | | 0.107 | |
| Abdominal subcutaneous fat (cm²) | AA | 353 (46) | | 379 (68) | |
| | AG/GG | 255 (7) | 38.4 | 295 (15) | 28.5 |
| | p | 0.038 | | 0.232 | |
| Abdominal visceral fat (cm²) | AA | 130 (16) | | 132 (19) | |
| | AG/GG | 95 (2) | 36.8 | 79 (4) | 67.1 |
| | p | 0.027 | | 0.006 | |

## 531

**Effects of high glucose treatment on the expression of TNFalpha and its receptors in human monocytes and lymphocytes in vitro**

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**Background and aims:** Although it is well known that diabetes is an inflammatory disease, the specific cellular and molecular mechanisms involved

are not fully resolved. Inflammatory cytokines and chemokines relevant to the pathogenesis of diabetes can be induced by hyperglycemia The effects of high glucose (HG) treatment on the expression TNFα and other inflammatory genes (IL-6, MCP-1, IL-1β etc.) *in vitro* on cell lines (THP-1, U937) has already been evaluated, however little is known the effect of HG on *in vitro* gene expression of the TNFα receptors (TNFR1 and TNFR2) on human monocytes and lymphocytes. Our aim is to evaluate the expression of TNFα and its receptors on human isolated monocytes and lymphocytes under different HG conditions and lipopolysaccharide (LPS) treatment.
**Materials and methods:** Monocyte-enriched fractions and non adherent cellular fractions (lymphocytes) from 6 healthy donors were incubated with 5 mM, 15 mM and 25 mM Glucose and with 1 μ/ml of LPS for 10 h. The mRNA levels of TNFα, TNFR1, TNFR2 and CD14 genes were analyzed by real time PCR. Statistical analysis was performed by using Mann-Whitney test in SPSS program version 11.
**Results:** In agreement to previous studies by other authors on human monocytes and cell lines, TNFα mRNA levels were increased up to 1,72 fold by glucose, however no changes were found on TNFR1 and TNFR2 mRNA. LPS enhanced the expression of TNFα and TNFR2 in monocytes (p=0,032 and p=0,008, respectively) but downregulates up to 50% the expression of TNFR1. In addition, in lymphocytes, LPS downregulates CD14 and TNFR1 gene expression.
**Conclusion:** This results show for the first time that the increment on the expression of TNFα mRNA levels due to acute HG treatment is not followed by an increased of expression of its ligands (TNFR1 and TNFR2).
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## 532

**TNF-α-induced signaling pathway leading to PAI-1 production and its cross-talk with renin-angiotensin system in human normal hepatocyte cell line**
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**Background and aims:** Tumor necrosis factor (TNF)-α and local activation of renin-angiotensin system (RAS) may contribute to insulin resistance and atherogenesis in obesity-associated metabolic syndrome. Circulating plasminogen activator inhibitor (PAI)-1 levels are highly dependent on insulin resistance. Although adipose tissue has been thought to be a potential contributor to plasma PAI-1 concentration, we extended the knowledge on PAI-1 by showing that TNF-α promotes PAI-1 mRNA expression and protein production by human hepatocytes, and that thiazolidinediones and statins are candidates to inhibit TNF-α-induced PAI-1 production ($38^{th}$ EASD & $18^{th}$ IDF congress). Here, we examined TNF-α-induced signaling pathway leading to PAI-1 production, expression of RAS components and cross-talk between TNF-α and RAS in hepatocytes.
**Materials and methods:** Simian virus 40 large-T (SV40-T) antigen immortalized normal human hepatocyte cell line, THLE-5b cells were incubated in PFMR-4 medium with 10% FBS. PAI-1 protein levels in the culture medium were determined by an enzyme-linked immunosorbent assay. PAI-1 mRNA levels were quantitated by real-time PCR, and expressed as relative expression ratios to an endogeneous control, 18S ribosomal RNA.
**Results:** (1) TNF-α stimulated PAI-1 protein production dose- and time-dependently. The optimal effect of TNF-α was found at 1.0 ng/ml for 24 hours, increasing PAI-1 release by 221 ± 3% (control 135 ± 1 vs TNF-α 299 ± 4 ng/ml, mean ± SEM, P<0.05). (2) TNF-α at 1.0 ng/ml induced a time-dependent increase in PAI-1 mRNA, with a maximum peak at 6 hours, corresponding to an increase by 1.7 fold. (3) A specific inhibitor for p38 mitogen-activated protein (MAP) kinase, SB 203580 (5 μM), and a protein kinase C (PKC) inhibitor, calphostin C (200 nM), and MAP kinase/extracellular signal-regulated kinase (ERK) kinase-specific inhibitor, PD98059 (40 μM), protein tyrosine kinase (PTK) inhibitor, genistein (10 nM), and NF-κB inhibitor, emodin (10 ng/ml) reduced TNF-α-induced PAI-1 production by 39%, 37%, 29%, 32% and 54%, respectively. (4) The THLE-5b cells expressed genes encoding the RAS components required for angiotensin II (AII) synthesis, angiotensinogen (AGT), angiotensin-converting enzyme (ACE) and AII receptor type 1 (AT1). AII receptor type 2 was not detected. (5) AGT mRNA was up-regulated time-dependently by TNF-α (1.0 ng/ml), whereas the expression of AT1 and ACE mRNAs were not altered. (6) AII at 50 nM increased PAI-1 mRNA and protein synthesis by THLE-5b cells. (7) AT1 blocker, olmesartan, at 0.1 and 1.0 μM reduced TNF-α-induced PAI-1 production by 34% and 60%, respectively.
**Conclusion:** TNF-α promotes PAI-1 mRNA expression and protein production via MAP kinase-, PTK- and NF-κB-dependent pathway by human hepatocytes, and may contribute to the impairment of the fibrinolytic system leading to development of atherosclerosis in insulin resistance. Hepatocytes express functional RAS components. TNF-α provokes increased expression of AGT, and AT1 blocker is a candidate to inhibit TNF-α-induced PAI-1 production.

## 533

**Leptin and TNF-α in subcutaneous fat tissue and in serum of healthy volunteers and of persons with Type 2 diabetes mellitus**
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**Background and aims:** Leptin and TNF-α are produced by adipose tissue as key serum mediators that interfere with insulin receptor signalling pathway; leptin takes part in regulation of food intake and metabolism; TNF-α leads to insulin resistance and obesity. In adipose tissue, concentrations of TNF-α correlate positively with the degree of obesity or hyperinsulinemia. TNF-α concentrations in fat are reduced in association with weight loss. Nevertheless, the precise role of leptin and TNF-α in the development of insulin resistance and associated metabolic disorders has not been clearly defined yet. The aim of this pilot study was to compare the concentrations of leptin and TNF-α in serum and in subcutaneous abdominal fat of young healthy non-obese volunteers and in persons with type 2 diabetes mellitus.
**Materials and methods:** Leptin and TNFα concentrations were measured in serum and subcutaneous fat homogenates of 12 healthy volunteers (2 men and 10 women) aged 28,7 ± 10 years (mean ± SD) and of 17 persons (11 men and 6 women) with type 2 diabetes aged 60 ± 11 years, diabetes duration 3 to 15 years, treated with complemetary doses of regular human insulin and, when necessary, with antihypertensive drugs, diuretics etc., using immunochemical ELISA method. Fat was obtained via aspiration of abdominal adipose deposits by means of needle Corecut and immediatelly put into fluid nitrogen and kept at -80 °C; the estimations were performed in the course of 12 months. Correlation between the body mass index (BMI) and the insulin resistance index QUICKI (1/[log serum insulin inμIU/ml + log glycaemia in mg/100 ml]), as well as the correlation between leptin and TNFα in both serum and adipose tissue, were estimated.
**Results:** When comparing the healthy volunteers and persons with type 2 diabetes, the former group was significantly more insulin resistant (index QUICKI was as low as 0,318 ± 0,04 compared with 0,369 ± 0,03 in the healthy volunteers, p≤0,01) and more obese (BMI 31,2 ± 3,8, compared with 21,6 ± 1,9 in the healthy volunteers, p≤0,001). The serum concentration of leptin was higher in persons with type 2 diabetes as compared to healthy volunteers (24,2 ± 17,8 ng/ml and 12,0 ± 7,1 ng/ml, respectively, p≤0,05), however, conversely, the serum concentration of TNFα was lower in persons with diabetes (1,4 ± 0,8 pg/ml), as compared to healthy volunteers (4,0 ± 3,1 pg/ml), p≤0,01. The concentrations of leptin and TNFα in subcutaneous fat homogenates were variable and did not differ singnificantly between healthy volunteers and persons with type 2 diabetes. BMI correlated with QUICKI in persons with diabetes, however, correlations between these parameters and concentrations of leptin and TNFα in serum and subcutaneous fat, respectively, were not found.
**Conclusion:** As expected, serum concentrations of leptin were significantly higher in persons with type 2 diabetes compared to younger healthy volunteers. On the other hand, serum concentrations of TNFα were surprisingly lower in persons with type 2 diabetes. In abdominal subcutaneous fat homogenates, the concentrations of leptin and TNFα were variable and no difference between healthy volunteers and persons with type 2 diabetes was found. Further studies are necessary.
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## 534

**Effect of exercise training and detraining on leptin, adiponectin and resting metabolic rate in inactive elderly men**
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**Background and aims:** Adiponectin and leptin, adipocyte-derived cytokines, are closely linked to insulin resistance and atherosclerosis. Regular exercise exerts favorable effects in the development of cardiovascular disease and type 2 diabetes mellitus. The aim of the study was to investigate

the responses of leptin, adiponectin and resting metabolic rate (RMR) during exercise training and detraining in elderly men.
**Materials and Methods:** Thirty-two inactive elderly men were randomly assigned to three 6-month supervised training programs of low (LI, no=8), moderate (MI, no=8) and high (HI, no=8) intensity. Eight subjects served as controls (CO, n=8). Six months after completing the 6-month training period all subjects were re-evaluated. Blood samples for the determination of leptin and adiponectin (RIA, Linco RI) were drawn at baseline, at the end of the training program and 6 months after detraining. RMR and $VO_2$max were determined at all time points.
**Results:** During the exercise period, BMI decreased in LI, MI and HI with a subsequent increase in the detraining period. Leptin decreased in the three groups ($p<0,01$), with levels at 6-months being significantly lower in HI group ($p<0,001$). In addition % decrease of leptin was independently associated with % decrease BMI and % increase RMR. During the detraining period leptin levels increased, but only HI group had significantly lower leptin levels compared to baseline. Adiponectin levels increased only in MI and HI groups ($p=0,03$ and $p=0,006$, respectively). % increase of adiponectin levels was significantly associated with both % decrease BMI and glucose levels ($p<0,05$). During the detraining period there was a non-significant decrease of adiponectin. However only HI group had significantly higher adiponectin levels compared to baseline.
**Conclusion:** Exercise training in elderly men induces significant changes in leptin and adiponectin levels, which are partly independent of changes in body mass index.

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**Leptin concentration is a more important marker to be closely related with insulin resistance than adiponectin**
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**Background and aims:** Obesity is associated with insulin resistance. Although two adipocyte-derived proteins, adiponectin and leptin, has been suggested to be the important mediators linking obesity and insulin resistance, there is no observation to simultaneously compare the usefulness of adiponectin and leptin as a marker for insulin resistance. We investigated the relationships between adiponectin or leptin and body fat distribution and insulin resistance.
**Materials and methods:** We measured plasma adiponectin, leptin, and visceral and subcutaneous fat thickness (VFT and SFT, respectively) by ultrasonography, and HOMR-IR index in 126 diabetic patients (84 men/42 women).
**Results:** The leptin ($r=0.368$, $P<0.001$), but not adiponectin ($r=-0.118$, P=NS), were significantly related to the homeostasis model assessment-insulin resistance index (HOMA-IR). Sex ($P<0.001$) and SFT ($P=0.01$), but not BMI, waist circumference, and VFT, contributed independently to leptin concentration. However, multiple logistic regression analysis revealed that only leptin concentration ($P<0.001$, 95%CI; 1.36, 2.65) was an important factor to predict the presence of metabolic syndrome based on the Adult Treatment Panel III criteria, independent of SFT. In contrast to leptin, there were no interrelationships between adiponectin concentration and adiposity or metabolic syndrome. A leptin concentration of 3.15 and 5.01 ng/mL, in men and women respectively, was chosen as the discriminator value to predict the presence or absence of the metabolic syndrome (specificity of 76% and sensitivity of 63% in men, and specificity of 78% and sensitivity of 80% in women).
**Conclusion:** Leptin concentration may be a better surrogate marker of insulin resistance or metabolic syndrome, as compared with that of adiponectin.

## 536

**Dramatic short-term efficiency of leptin replacement treatment in minimizing the metabolic complications in children with congenital lipoatrophy (cla)**
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Congenital lipoatrophy is a recessive congenital disease marked by the complete lack of subcutaneous adipose tissue. Severe and life-threatening metabolic complications, such as insulin resistance, dyslipidemia, NASH are seen as a consequence to the profound defect in plasma leptin. The aim of the study was to assess the efficiency of a replacement leptin treatment on the metabolic complications in non-diabetic children with CLA. Recombinant methionly human leptin (r-metHuLeptin) was administered subcutaneously for 4 months with increasing dosages up to 0.03 mg/kg of lean mass daily in seven children (6 boys and 1 girl; age 2.4–13.5 yr.; 3 prepubertal) in whom basal plasma leptin was <3 ng/ml. Injections were well tolerated; no serious adverse effect was observed and there was no acceleration of pubertal development. The metabolic effects are presented in the table below (fat mass was measured using DEXA and liver volume by CT-scan; insulin sensitivity was measured as the peripheral glucose uptake -M- under a euglycemic hyperinsulinemic clamp at 80 mU/kg/min. in children <6 yr.) (table). Treatment of children with CLA and reduced leptin levels with replacement doses of r-metHuLeptin had significant effects on multiple metabolic parameters. In two children<6 yr. of age, fasting insulin decreased from 101.7 ± 14.82 to 42.9 ± 40.2 pmol/l. Altogether, insulin and triglycerides levels decreased with treatment and normalized in 5/7 children. In conclusion, leptin replacement is able not only to improve but also to correct metabolic consequences of CLA with leptin deficiency at an early stage attesting for the important effects of leptin on key organs of metabolism.

Table

| | Before | After | p (paired analyses) |
|---|---|---|---|
| Weight (kg) | 37.7 ± 15.8 | 37.41 ± 16.61 | 0.61 |
| Waist circum.(cm) | 65.44 ± 7.43 | 61.67 ± 6.75 | 0.1 |
| Fat mass (kg) | 2.04 ± 0.86 | 2.41 ± 2.08 | 0.21 |
| Fasting Plasma Glucose (mmol/l) | 4.46 ± 0.38 | 4.46 ± 0.29 | |
| Fasting Insulin (pmol/l) | 136.2 ± 84.18 | 100.86 ± 180.28 | 0.2 |
| M (mg/min/kgFFM) (n=4) | 7.21 ± 4.33 | 12.2 ± 2.64 | 0.018 |
| Cholesterol (mmol/l) | 4.38 ± 1.2 | 3.71 ± 09 | 0.007 |
| Triglycerides (mmol/l) | 2.87 ± 1.28 | 1.65 ± 1.17 | 0.009 |
| Liver volume (l) | 4.53 ± 1.43 | 3.22 ± 0.89 | 0.002 |

## 537

**The relationship between insulin resistance and fasting leptin in subjects with risk factors of diabetes mellitus Type 2 in Belarus**
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**Background and aims:** Leptin, the adiposity hormone, is increased in obese individuals, suggesting resistance to its effect. Differences have been observed in the relationship between leptin and metabolic perturbations in glucose homeostasis. The purpose of this study was to investigate the possible associations between leptin and fasting insulin (FI), proinsulin (PI) and insulin resistance (the HOMA model) in patients with impaired glucose tolerance (IGT), impaired fasting glucose (IFG) and newly diagnosed diabetes mellitus type 2 (DM 2) by 1999 WHO criteria.
**Materials and methods:** 100 patients (32 m, 68 f) were divided in four groups: IGT-group: 32 patients (10 m, 22 f) mean age 49.7 ± 12 years, mean body mass index (BMI) 28.1+/–4.8 kg/m$^2$, waist-to-hip ratio (WHR) 0.83+/–0.07; IFG-group: 14 patients (6 m, 8 f), age 48.7+/–13.7, BMI 28.4+/–4.9 kg/m$^2$, WHR 0.83+/–0.02; newly diagnosed DM 2 group: 24 patients (7 m, 17 f), age 49.9+/–11.3, BMI 29.3+/–5.4 kg/m$^2$, WHR 0.84+/–0.06; normal glucose tolerance (NGT) group: 30 patients (9 m, 21 f), age 47.3+/–12.4, BMI 27.8+/–4.2 kg/m$^2$, WHR 0.83+/–0.05. We measured fasting plasma glucose (FPG), 2-hour plasma glucose concentrations (2-h PG) following a 75-g oral glucose tolerance test. Fasting serum leptin, PI and FI levels were detected by sensitive and specific ELISA. Index HOMA-R = fasting glucose [mmol/l] × fasting insulin [μU/ml]/22.5 were used as the index of insulin resistance. HOMA-R =or> 2.7 were considered as insulin resistance. Comparisons among groups were performed with ANOVA.
**Results:** In all studied groups serum leptin levels were higher in females than in males and correlated significantly to BMI and WHR. The main novel finding was that median serum leptin was significantly higher in diabetic subjects (47.7 ng/ml) compared to IGT-group (35.4 ng/ml), IFG-group (16.4 ng/ml) and NGT-group (23.7 ng/ml) ($p<0.05$ for all groups), although BMI did not differ between groups. Fasting insulin levels and HOMA-R also were higher in diabetic subjects than in other groups (mean+/-SD): fasting insulin in newly diagnosed DM 2 group was 15.9+/–3.9 μU/ml, in IGT-

group 11.3+/–4.7 μU/ml, in IFG-group 9.9+/–2.9 μU/ml and in NGT-group 8.6+/– 2.5 μU/ml; HOMA-R in newly diagnosed DM 2 group was 4,6+/–1,1, in IGT-group 2.7+/–1.2, IFG-group 2.4+/–0.7 and NGT-group1.8+/–0.6 ($p<0.05$ for all groups). Proinsulin concentrations did not differ significantly between groups. But patients with DM 2 and BMI more than 30 kg/m$^2$ had higher proinsulin levels than diabetic with BMI <30 kg/m$^2$ ($p<0.05$). After further adjustment for BMI and WHR, leptin levels remained significantly correlated in diabetic and IGT subjects with the FI ($r=0.56$ $p<0.01$ vs $r=0.39$ $p<0.05$ respectively), HOMA-R ($r=0.52$ $p<0.01$ vs $r=0.43$ $p<0.05$ respectively) and were not correlated with PI. In subjects of NTG-group leptin concentration correlated with the HOMA-R only in men with BMI =or> 25 kg/m$^2$ ($r=0.91$ $p<0.01$). In subjects of IGT-group and IFG-group FP levels positively correlated with FI ($r=0.34$ $p<0.05$ vs $r=0.61$ $p<0.05$ respectively) and HOMA-R ($r=0.42$ $p<0.05$ vs $r=0.6$ $p<0.05$ respectively).
**Conclusion:** Subjects with risk factors of diabetes mellitus type 2 in Belarus have higher leptin and insulin levels and HOMA-R. Proinsulin concentration has no influence on leptin levels but associated with serum insulin levels and insulin resistance in subjects with IGT and IFG.

## 538

**Differentiated IRS-2 (-/-) brown adipocytes develops a caveolin-dependent glucose transport**
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**Background and aims:** Insulin induces glucose transport in a PI3 quinase / IRS-2/PKC-z-dependent manner in neonatal brown adipocytes. Accordingly, IRS-2 -/- fetal brown adipocytes fail to induce glucose transport in response to insulin. However, those cells trigger full differentiation under certains conditions. Here, we investigate the role of brown adipocyte differentiation in the onset of a caveolin-dependent glucose transport pathway.
**Materials and Methods:** Neonatal brown adipocytes, with or without IRS-2, were submitted to the differentiation protocol for two days before confluence in the presence of T3 and insulin, three days after confluence in the presence of T3, insulin, IBMX, dexamethasone and indometacin and, finally, two days further with T3 and insulin.
**Results:** Insulin stimulated glucose transport in wild-type but failed to in IRS-2 –/– neonatal brown adipocytes. Both kind of brown pre-adipocytes express at very low levels essential genes such as Glut-4, IRS-3, C/EBP-alpha, UCP-1 and caveolin. After differentiation, both kind of cells show a full lipid content and also markedly increase the expression of caveolin, IRS-3, Glut-4 and C/EBP-alpha. Under these conditions, insulin increased glucose transport in wild-type cells as compared to undifferentiated cells in a PI 3 kinase-dependent manner. IRS-2 –/– differentiated cells induce glucose transport in response to insulin, this effect being more pronounced in cells overexpressing exogenous IRS-3. In both cases, the enhancement of glucose transport in response to insulin was independent of a PI 3 kinase specific inhibitor (wortmannin) and may imply a cbl/cap signaling pathway.
**Conclusion:** Our results indicate that differentiated brown adipocytes increase glucose transport in response to insulin as compared to preadipocytes. More importantly, cells lacking IRS-2, with or without exogenous IRS-3, induce a PI 3 kinase-independent glucose transport in response to insulin upon differentiation, in parallel to the expression of caveolin and endogenous IRS-3.

# PS 35
# Insulin action in adipocytes

## 539

**Phosphorylation of GATA-2 transcription factor regulates preadipocytes transition to adipocytes**
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**Background and aims:** GATA transcription factors have been recently involved in adipogenesis. We identified in GATA2 transcription factor an Akt consensus motif (RNRKMS395).
**Materials and methods:** We prepared three constructs encoding for GATA2 (WT), GATA2A (ser395ala, which mimicks a GATA2 that lack phosphorylation site) or GATA2D (ser395asp, which mimicks a GATA2 that should be always phosphorylated). We used HEK293 cells to study phosphorylation upon insulin stimulation, 3T3F442A preadipocytes to assess role on adipogenesis in vitro and SCID mice to study the role of serine 395 phosphorylation on adipogenesis in vivo.
**Results:** We observed that ser395 was significantly phosphorylated by Akt, in vitro. Insulin stimulation of HEK293 cells significantly increased GATA2 ser395 phosphorylation, while pretreatment of cells with wortmannin blocked it, indicating that GATA2 is phosphorylated at ser395 by insulin, through a PI3K/Akt dependent pathway. Moreover, we found that ser395 phosphorylation is crucial for the regulation of GATA2 DNA binding activity, which takes part in GATA2 transcriptional activity. It has been suggested that GATA2 regulates adipocyte differentiation through control of the preadipocyte-adipocyte transition. Retroviral infection of 3T3F442A preadipocyte with GATA2, GATA2A or GATA2D has shown that GATA2A impaired adipocyte differentiation (mimicking GATA2 effect), while GATA2D mutant restored the cells ability to differentiate, through modulation of PPAR-gamma expression. To investigate if GATA2 Ser395 phosphorylation is also involved in the correlation between adipose tissue and inflammatory processes, we have measured the phagocytosis of proliferating preadipocytes, that is related to the macrophage-like cells activity. We found that the phagocytic activity is 30% increased ($p<0.01$) in presence of GATA2 or GATA2A, compared to GATA2D. Adipose cells are known to secrete anti- and pro-inflammatory cytokines. We observed that 3T3F442A-GATA2A cells show a significant upregulation of TPO (75%) and MCP-1 (60%) expression, if compared to GATA2D. Furthermore, 3T3F442A-GATA2A produce growth factors like GM-CSF and IL-4, absent in 3T3F442A-GATA2D infected cells. To evaluate the significance of ser395 phosphorylation in an in vivo model we injected SCID mice with 3T3F442A-GATA2, 3T3F442A-GATA2A and 3T3F442A-GATA2D cells, and maintained mice on high fat diet for 8 weeks. Results from this experiment suggest that 3T3F442A-GATA2D maintain their adipogenic ability in vivo, while 3T3F442A-GATA2A show a decreased differentiation ability, similarly to in vitro conditions.
**Conclusion:** Our data suggest that ser395 phosphorylation of GATA2, modulated by insulin action, can contribute to regulate preadipocytes differentiation respectively into adipocytes or into macrophage-like cells.
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**Adipogenesis in human preadipocytes is blocked by tissue non-specific alkaline phosphatase inhibitors**
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**Background and aims:** Alkaline phosphatase (AP) exits as 4 different isozymes termed tissue non-specific
(TNAP), germ cell, placental and intestinal. The last 3 of these isozymes are termed tissue specific AP (TSAP). All 4 isozymes are the products of separate genes. AP may play a role in cellular differentiation and it is known to be present in human and rodent adipocytes. Therefore, the aim of this study was to determine whether AP plays any role in the adipogenic process in human preadipocytes.
**Materials and methods:** Adipose tissue was obtained from 12 human breast reduction operations and preadipocytes isolated by collagenase digestion

and centrifugation of the adipocyte isolate. Preadipocytes were induced to differentiate by culturing in differentiation medium containing insulin, IBMX, triiodothyronine and hydrocortisone. Cells were cultured in the presence or absence of the TNAP inhibitors levamisole and histidine and the TSAP inhibitor, Phe-Gly-Gly. Adipogenesis was assessed at 0 and 12 days post induction of differentiation using the triglyceride-specific dye oil red O. Intracellular AP activity was measured at the same time points. Intracellular localisation of AP was determined using a fluorescent AP substrate.
**Results:** AP activity was 37.9 ± 8.0 mU/mg protein in undifferentiated cells and after differentiation increased 5.1 ± 1.3 fold in the absence and 8.9 ± 2.8 fold ($p=0.06$) in the presence of levamisole i.e. levamisole did not inhibit AP activity. However, when AP was isolated from the preadipocytes levamisole produced a 49.6 ± 1.3% inhibition ($p<0.05$) of AP activity. Adipogenesis increased 1.95 ± 0.11 fold in the absence but only 1.36 ± 0.06 fold ($p<0.001$) in the presence of levamisole. There was a 4.2 ± 2.2 fold increase in AP activity in the absence and a 0.51 ± 0.46 fold ($p<0.05$) decrease in the presence of histidine. Adipogenesis increased 2.09 ± 0.35 fold in the absence of histidine but only 1.22 ± 0.30 fold ($p<0.05$) in the presence of histidine. Phe-Gly-Gly had no effect on AP activity or adipogenesis. Using fluorescent microscopy in the presence of an AP substrate that gives rise to a fluorescent end product, AP activity was localised to the triglyceride-containing droplets of the cell.
**Conclusion:** Levamisole can inhibit AP activity in cell free extracts but not in intact preadipocytes and can block adipogenesis independently of AP, possibly via its known adrenergic or cholinergic effects. AP activity and adipogenesis are inhibited by histidine but not by Phe-Gly-Gly. This is the first study to show that AP is present in human preadipocytes and that TNAP inhibitors can block adipogenesis. The data from the inhibitor studies and the localisation of AP activity to the lipid-containing droplets of human preadipocytes strongly suggest that TNAP is involved in the regulation of adipogenesis in humans. Thiazolidinediones are known to increase both adipogenesis and insulin sensitivity and therefore TNAP may be a new target for therapeutic modulation of weight gain and/or insulin sensitivity.

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**The histone code of mature adipocyte gene activation**
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**Background and aims:** White adipose tissue (WAT) is an endocrine organ that plays a central role in the regulation of energy balance and mediates physiological and pathological processes through the secretion of a number of bioactive molecules collectively known as adipocytokines. Dysregulation of WAT causes obesity, which may lead to chronic disorders such as diabetes and cardiovascular diseases. Adipocyte differentiation is a complex process regulated both hormonally and transcriptionally. The nuclear receptor peroxisomal proliferator-activated receptor γ (PPARγ) acts as a master regulator of adipogenesis, and its activity is regulated by cofactors such as the histone acetyltransferase CBP and the histone deacetylase HDAC1. These data suggest that the regulation of covalent histone modifications plays a key role in the establishment of the pattern of gene expression of mature adipocytes. We are interested in studying the histone post-translational modifications taking place throughout adipocyte differentiation and their role in the process of adipogenesis.
**Material and methods:** Murine 3T3-L1 preadipocytes were differentiated by incubation in the presence of a mix containing 3-isobutyl-1-methylxanthine, dexamethasone and insulin. The cells were used at different time points during the differentiation process. Post-translational modifications of histones as well as transcription factor binding to the promoters of interest were studied using chromatin immunoprecipitations (ChIPs) as described elsewhere.
**Results:** We used ChIPs to examine the post-translational modifications of histones which take place during the process of adipogenesis. We focused on later markers of adipogenesis, such as adiponectin, leptin, glycerolphosphate dehydrogenase (GPDH) and glucose transporter GLUT4. The promoters of most of the genes studied were not acetylated nor methylated in preadipocytes, where they are not expressed. The first modification detected at the promoter regions of the genes of interest was methylation of lysine K4 of histone H3. This methylation event is a required first step in gene activation and is shortly followed by histone H3 hyperacetylation. In fact, histone H3 hyperacetylation at the promoters displayed a better correlation with gene expression than methylation at the same regions, as some genes were methylated but not acetylated in preadipocytes, well before their expression started. Interestingly, H3 methylation but not acetylation was extended to the coding regions of the genes studied upon transcription initiation. Thus, promoter hyperacetylation and coding region methylation correlated with transcriptional activity of mature adipocyte markers.
**Conclusion:** A cascade of histone modifications takes place during adipogenesis and is necessary for the establishment of a permissive chromatin state in the promoters of key adipogenic markers. The acquisition of a detailed knowledge of the epigenetic events regulating adipogenesis would allow us to manipulate adipocyte differentiation and function, ultimately leading to the discovery of new therapeutic targets for the treatment of obesity and diabetes.
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## 542

**Indications of a secondary role for IRS depletion in the mechanism behind impaired glucose uptake in primary rat adipocytes**
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**Background and aims:** Long-term treatment (24 h) of isolated rat adipocytes with high glucose (15 mM) and insulin ($10^4$μU/ml) decreases both basal- and insulin-stimulated glucose uptake capacity markedly and concomitantly the amount of insulin receptor substrate-1 and -2 (IRS-1/2) are downregulated. The aim of the present study was to investigate the mechanisms behind the downregulation of IRS-1/2 and its significance for the impaired glucose uptake capacity.
**Materials and methods:** Isolated epididymal rat adipocytes were cultured (24 h) under four different conditions: low or high glucose (5 mM/15 mM glucose) with or without high insulin ($10^4$μU/ml) concentration. mRNA was extracted and semiquantitative PCR was performed. Isolated adipocytes were also cultured in 5 mM or 15 mM glucose and high insulin with or without the proteasome inhibitor lactacystin (1 h pretreatment) or the protein synthesis inhibitor cycloheximide. Protein was extracted and immunoblotting was performed. Basal and insulin-stimulated glucose uptake capacity was assessed in intact cells.
**Results:** 24 h pretreatment with high glucose and/or insulin did not alter mRNA expression of β-actin, insulin receptor, IRS-1, PI3-K, or PKB compared to the control situation. However, high glucose and insulin induced a marked decrease in IRS-2 mRNA expression. Adipocytes cultured in 5 mM glucose with cycloheximide showed a marked decrease in IRS-2 but not IRS-1 levels. Despite this the glucose uptake capacity was unaffected. Preliminary data indicate that cycloheximide in 15 mM glucose and high insulin lead to further reduction of the level of IRS-1, compared to 15 mM glucose and high insulin alone, but did not worsen the impaired glucose uptake capacity. Lactacystin pretreatment reduced the levels of both IRS-1/2, however the reduction of IRS-1 was smaller in cells cultured in 15 mM glucose and high insulin. Lactacystin pretreatment alone impaired glucose uptake capacity, probably due to the observed PKB depletion, but the additive effect of high glucose and insulin concentrations were still observed. 6 h pretreatment with high glucose and insulin concentrations were followed by up to 24 h incubation in 5 mM glucose in an attempt to reverse the impaired glucose uptake. After 12 h a partial recovery was seen that was not improved upon further incubation periods, however these observations were not associated with any improvements of either IRS-1 or -2 levels.
**Conclusion:** We conclude that gene expression explain the reduced IRS-2 levels upon long-term treatment of adipocytes with high glucose and insulin. However, cycloheximide-induced depletion of IRS-2 did not affect the glucose uptake capacity. The lactacystin induced reduction of IRS-1 was greater in 5 mM glucose, still the additive effect on the glucose uptake capacity impairment of high glucose and insulin concentrations were still observed. Partial recovery in glucose uptake did not include any improvements in the levels of IRS-1 or -2. Taken together these preliminary data indicate a secondary role of IRS-1/2 depletion in the mechanism behind impaired glucose uptake.

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**Suppression of endogenous PPARγ-target gene expression and adipogenic potential of 3T3-F442 preadipocytes by RIP140 corepressor**
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**Background and aims:** PPARγ, a steroid hormone receptor, plays an important role as a master transcription factor in glucose homeostasis as well as

in the modulation of atherosclerosis. PPARγ interacts physically with its ligands such as anti-diabetic compounds of thiazolidinediones (TZDs) to release corepressors and recruit coactivators. This coordinated event eventually leads to its target gene expression. Recently, the receptor interacting protein 140 (RIP140) has been shown to interact with the ligand-associated steroid hormone receptors including RARs, TR, and PPARs, and suppress the activity of the receptors. Therefore, this study examined whether or not RIP140 suppresses PPARγ-target gene expression and adipogenesis *in vivo*.
**Materials and methods:** Transient transfection assays were performed to test effect of RIP140 on the ligand-dependent transcriptional activity of PPARγ. Recombinant retrovirus of RIP140 was transduced to NIH3T3 cells to test effect of RIP140 on the expression of the endogeneous PPARγ-target genes in PPARγ/RIP140-coexpressing NIH3T3 cells. Northern blot analysis was performed to survey expression of RIP140 and the adipogenic marker mRNAs during the adipogenic differentiation of 3T3-F442A cells. Recombinant retrovirus of RIP140 was transduced to 3T3-F442A cells to test effect of RIP140 on the adipogenic potential of the preadipocytic 3T3-F442A cells.
**Results:** The results showed that RIP140 potently suppressed the ligand-associated PPARγ activity in ARF6, a transcriptional complex, which is essential for the adipocyte-specific expression of the PPARγ target genes. This decreased PPARγ activity was associated at least in part with the histone deacetylases. The transfection studies using a GAL4DBD/PPARγ hybrid construct suggested that PPARγ rather than its associated proteins was directly mediated in the suppression of the ligand-associated PPARγ activity by RIP140. Surprisingly, the RIP140 mRNA level appeared to be induced at the later stage of adipogenic differentiation. Importantly, Northern blot analysis and the Oil Red O staining studies revealed that the overexpressed RIP140 suppressed not only the endogenous PPARγ-target gene expression in the PPARγ and RIP140-stably coexpressing NIH3T3 cells, but also the adipogenic potential of the preadipocytic 3T3-F442A cells.
**Conclusion:** Overall, these results suggest that RIP140 suppresses the adipogenic potential of adipocytic cells *in vivo*, most probably by the suppression of the endogenous PPARγ-target genes. Finally a model was constructed based on the RIP140 action in order to better understand the molecular mechanisms involved in the paradoxical action of PPARγ-mediated TZDs; TZDs activate PPARγ not only for adipogensis possibly leading to the development of obesity-associated insulin resistance, but also to improve the obesity-associated insulin resistance.

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**Oleylethanolamide inhibits glucose uptake by serine phosphorylation of GLUT-4 in rat adipocytes**

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**Background and aims:** Oleylethanolamide (OEA) is a lipid mediator that inhibits food intake and body weight gain, and exhibits hypolipemiant actions. OEA exerts its anorectic effects peripherally through the stimulation of c-fibers. Recent data show that OEA is also an activator of peroxisome proliferator receptor alpha. OEA is synthesized in the intestine in response to feeding, increasing its levels in portal blood after the meal. Moreover, OEA is produced by the adipose tissue, suggesting a role in the function of the adipocyte. Recently we have found that OEA inhibits glucose uptake in rat adipocytes and impairs glucose tolerance in vivo. In the present work we sought to study the mechanism of action of OEA inhibiting glucose uptake in rat adipocytes.
**Materials and methods:** Glucose uptake was studied in isolated epididymal rat adipocytes by using 2-deoxy-D-[3H]glucose. GLUT-4 translocation was assessed by immunoblot of adipocyte plasma membrane fraction. GLUT-4 phosphorylation was determined by anti-phosphoserine immunoblot of GLUT-4 immunoprecipitates. Pharmacological inhibitors of different kinases were employed to study the mechanism of OEA action.
**Results:** We found that 10 min preincubation with OEA inhibited 25% basal and 50% insulin stimulated glucose uptake in isolated adipocytes without preventing GLUT-4 translocation to the plasma membrane. GLUT-4 in the plasma membrane was serine phosphorylated in response to OEA, however. The maximal effect was achieved at 1 micromolar OEA. The related compounds palmitylethanolamide and oleic acid had no effect, suggesting a specific mechanism whereby OEA inhibits glucose transport activity and promotes GLUT-4 serine phosphorylation. The effects of OEA on GLUT-4 phosphorylation, as well as the inhibition of glucose transport was prevented by blocking p38 MAPK with SB203580.
**Conclusion:** These results suggest that the lipid mediator OEA inhibits insulin-stimulated glucose uptake in adipocytes, by decreasing glucose transport activity. This effect seems to be mediated by serine phosphorylation of GLUT-4 via p38 MAPK pathway. Therefore, OEA may contribute to insulin resistance in adipose tissue.
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**CGR19 binds to IRS proteins and regulates insulin-dependent glucose uptake and proliferation in vitro**

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**Background and aims:** CGR19 is expressed at moderate levels in cells with functional p53. It has been shown to inhibit the growth of several cell lines. We have identified CGR19 in a Yeast 2-Hybrid screen, as an IRS-1 and IRS-2 binding protein. Our objective was to investigate if CGR19 could play a role in the insulin signalling cascade.
**Materials and methods:** We have used an adenoviral construct *(Adv-CGR19)* to overexpress full-length CGR19 in CHO-IR cells or in 3T3-L1 adipocytes. Transfections with *Adv-GFP, Adv-β-GAL* or with empty vector were used as controls. The effect of CGR19 on insulin-induced proliferation of CHO-IR cells was assessed by measuring 3H-Thymidine incorporation. The role of CGR19 in insulin-regulated glucose transport was determined in 14C-2-deoxy-D-glucose uptake assays. Western blotting was used to assess phosphorylation of downstream signalling components MAPK and Akt/PKB.
**Results:** CGR19 had a mildly inhibitory effect on insulin-induced proliferation in CHO-IR cells. Overexpression of CGR19 in 3T3-L1 adipocytes increased the baseline rate of glucose uptake. Insulin-induced glucose uptake was also higher in *Adv-CGR19*-transfected adipocytes compared to controls, but only when insulin exposure was long term (2 hours, rather than 30 minutes). Increasing doses of *Adv-CGR19,* but not control vector, increased Akt/PKB phosphorylation in the absence of insulin.
**Conclusion:** Our results suggest that CGR19 is a positive regulator of glucose transport in 3T3-L1 adipocytes. Overexpression of CGR19 seems to prolong insulin action in adipocytes but has no increasing effect on glucose uptake in the short term. This effect might be mediated by Akt/PKB but it remains to be determined if IRS-dependent signalling is involved. Surprisingly, we found only a minor effect of CGR19 on proliferation.

# PS 36
# Intramyocellular lipids

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**Discontinuation of rosiglitazone-treatment in male Zucker Diabetic Fatty rats: effects on intramyocellular lipids and glucose homeostasis**

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**Background and aims:** PPARγ agonists, like rosiglitazone (RGZ), are applied as insulin sensitizers in type 2 diabetic patients. They act by influencing differentiation and distribution of adipocytes and by modulating lipid and glucose metabolism. RGZ therapy improves plasma lipid profile and insulin sensitivity (IS). The amelioration of glucose homeostasis has been shown to correlate closely with a reduction of intracellular lipid stores in skeletal muscle (IMCL) and liver (HepCL). Recent studies in Zucker Diabetic Fatty (ZDF) rats treated with RGZ have demonstrated an increase in IS concomitant with a clear reduction of IMCL and HepCL. The aim of the present study was to monitor the effects on IMCL, HepCL and plasma parameters after discontinuation of RGZ treatment.
**Materials and methods:** Obese male ZDF rats (n = 16) were treated from their 6th week of age on with RGZ (3 mg/kg/day). At 16 weeks of age, 8 animals were taken off treatment. Additional groups of obese and lean ZDF rats (n = 8 each) served as controls. Body weight, metabolic plasma parameters, IMCL and HepCL were regularly determined. IMCL in tibialis muscle (TIB) and HepCL were measured by $^1$H-NMR-spectroscopy.
**Results:** RGZ treatment led to strong weight increase, specifically of fat tissue. Normoglycemia was maintained thus preventing the development of diabetes, while plasma levels of triglycerides (TG) and free fatty acids (FFA) were significantly reduced as compared to obese controls. IMCL was significantly decreased. After discontinuation of RGZ treatment, animals developed overt diabetes within one week with hyperglycemia and rising plasma lipids. Manifestation of diabetes led to a sixfold IMCL and a fivefold HepCL rise 10 days after discontinuation of treatment. Thereafter, a slow continual decrease of these parameters as well as of bodyweight was observed.
**Conclusion:** In the insulin-resistant prediabetic young ZDF rat, RGZ ameliorated glucose and lipid homeostasis in terms of plasma parameters as well as of stored lipids in muscle and liver. Thereby manifestation of overt diabetes was prevented. When treatment was discontinued, diabetes ensued within a few days with increased levels of lipid in plasma as well as in muscle and liver („lipid overflow"). Discontinuation of RGZ treatment immediately led to overt diabetes accompanied by a rearrangement of lipid stores from adipose to muscle and liver tissue. The subsequent decline of IMCL and HepCL as well as of body weight is indicative of a catabolic lipid metabolism within the setting of diabetes. The effects observed on discontinuation of RGZ treatment demonstrate the key role played by lipids and in particular by ectopic lipid deposition in the development of IR and diabetes. RGZ acts by increasing the number of adipocytes and generating large adipocytic lipid stores. However, the insulin-sensitizing effect depends on permanent treatment.

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**Skeletal muscle triglyceride accumulation is associated with protein kinase C activation in insulin-resistant non-obese hypertriglyceridemic rats**

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**Background and aims:** Several studies in the obese humans and animals have demonstrated a relationship between muscle fat content and insulin resistance but in some studies, no relation was found between lipid accumulation and insulin sensitivity independent of obesity. The aim of the study was to compare the muscle triglyceride content in non-obese hereditary hypertriglyceridemic (HHTg) and normotriglyceridemic control rats (C) and to investigate whether it is related to serum free fatty acid levels, muscle insulin sensitivity and protein kinase C (PKC) expression and cellular localization. HHTg rats, originating from the Wistar strain, exhibit most symptoms of metabolic syndrome: hyperinsulinemia, impaired glucose tolerance, tissue resistance to insulin action and mild hypertension.
**Materials and methods:** The experiments were carried out in adult in 18-months old male HHTg rats and Wistar rats were used as controls. All the animals were fed standard laboratory diet. Tissue sensitivity to insulin action was measured in vitro by incubating musculus soleus in Krebs-Ringer buffer without or with insulin (250 μU/ml) according to $^{14}$C-U-glucose incorporation into glycogen. The protein amount of PKC θ, which is the major muscle isoform of serine/threonine kinase, in cytosol and membrane fractions of skeletal muscle was determined by Western blotting.
**Results:** The weight of epididymal adipose tissue, when related to body weight (HHTg: 380 ± 12 g, C: 461 ± 24 g, $p < 0.02$) was not different between HHTg and control rats (1.19 ± 0.14 vs 1.26 ± 0.09 g /100 g b.wt., N.S.). Compared with controls, triglyceridemia (1.89 ± 0.22 vs 0.91 ± 0.05 mmol/l, $p < 0.05$), serum free fatty acid levels (0.94 ± 0.07 vs 0.67 ± 0.05 mmol/l, $p < 0.001$) were higher in HHTg rats. In musculus soleus, triglyceride content was markedly elevated in HHTg rats as compared with controls (6.36 ± 0.61 vs 3.67 ± 0.40 μmol/g w.wt., $p < 0.001$). Insulin induced increase in $^{14}$C-U-glucose incorporation into skeletal muscle glycogen was lower in HHTg rats than in controls (54% vs 97%, $p < 0.02$). Study of insulin signaling indicated that there was a significantly higher protein content in of PKC θ isoform (by 55%, $p < 0.02$) in membrane fraction and lower protein content of PKC θ (by 36%, $p < 0.05$) in cytosol fraction from skeletal muscle in HHTg rats than in the control group.
**Conclusion:** Data suggested that elevated levels of circulating triglycerides and free fatty acids together with excessive deposition of triglycerides in the skeletal muscle and PKC θ activation may be involved in the mechanism of insulin resistance which is not associated with obesity.
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**Correlation between insulin sensitivity indizes derived from glucose clamp studies and IMCL content in different rat models of insulin resistance**

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**Background and aims:** Increased intramyocellular lipid (IMCL) content is supposed to be causally related to impaired insulin-stimulated glucose uptake and has thus been proposed as a surrogate marker for insulin resistance (IR) in humans. The glucose clamp technique is recognized as „gold standard" method for the assessment of insulin sensitivity (IS). An inverse correlation between IMCL content and IS as measured by the glucose clamp was found in humans, whereas only a few validation studies have been performed in animal models. Aim of this study was to investigate the putative negative interrelation between IS in terms of glucose disposal (GD) as well as exogenous glucose infusion rates (GIR) and IMCL content in the tibialis (TIB) and the soleus (SOL) muscle in different rat models of IR
**Materials and methods:** Diet-induced IR (fructose and „cafeteria"-fed Wistar rats) as well as genetic disease models (female obese ZDF rats exhibiting severe IR while maintaining normoglycemia, male obese ZDF rats developing overt diabetes, and their respective lean littermates) were used. One group of female lean ZDF rats was fed a high-fat diet. Subgroups of obese ZDF rats underwent long-term treatment with rosiglitazone (RGZ, 3 mg/kg/day). Glucose clamp studies were performed in anaesthetized animals. The study design consisted of a 2-h basal period followed by a 2-h euglycemic-hyperinsulinemic clamp, with an additional constant infusion of [U-$^{13}$C] glucose. Endogenous glucose production (EGP) was calculated from the enrichment of labelled glucose ([U-$^{13}$C] /[$^{12}$C] glucose-ratio). Calculations of IS indices were carried out during steady state conditions: GIR was calculated as the mean glucose infusion rate to maintain euglycemia, GD was computed as the sum of GIR and EGP. In diabetic animals, basal GD values were corrected for urinary glucose loss. IMCL levels were determined in TIB and SOL by $^1$H-NMR-spectroscopy at the day prior the clamp.
**Results:** IMCL in both muscles was elevated in diet-induced models compared to controls as well as in obese ZDF rats compared to their lean littermates. The IS indices, i.e. GIR and GD, were deteriorated both in diet-fed animals and in genetic models of IR. Excluding RGZ treatment, IMCL in TIB correlated better with both parameters for IS than IMCL in SOL. Comparing GD and GIR, the correlation with IMCL proved to be significant merely when using GD as IS index. The best fit between IMCL and IS indices was obtained using TIB and GD ($r^2 = 0.72$, $p < 0.01$).Overtly diabetic rats exhibited comparatively low IMCL levels due to their catabolic lipid metabolism: exclusion of this group improved $r^2$. RGZ-treated animals displayed increased GIR and GD, while exhibiting similar or even higher IMCL compared to diabetic controls.
**Conclusion:** The validity of TIB IMCL as marker for IS was confirmed in diet-induced models as well as in genetic ones – except for inconsistent ICML values measured in RGZ-treated rats, which might be attributed to

compound-inherent effects on fat distribution and body composition, impeding precise IMCL determination. In summary, TIB IMCL represents a valid biomarker for IS in various rat models of IR implying the advantage of repeated non-invasive measurements in one individual rat.

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**Intramyocellular lipid content in Type 2 diabetes patients compared to overweight sedentary controls and highly trained endurance athletes**

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**Background and aims:** Recent evidence suggests that intramyocellular lipid (IMCL) accretion is associated with obesity and the development of insulin resistance and/or type 2 diabetes. However, trained endurance athletes are markedly insulin sensitive, despite an elevated mixed muscle lipid content. In an effort to explain this metabolic paradox, we compared muscle fibre type specific IMCL storage between populations known to have elevated IMCL deposits.
**Materials and methods:** Immunofluorescence microscopy was performed on muscle biopsies obtained from 8 highly trained endurance athletes, 8 type 2 diabetes patients and 8 overweight, sedentary men following an overnight fast.
**Results:** Mixed muscle lipid content was substantially greater in the endurance athletes (4.0 ± 0.4% area lipid stained) compared to the diabetes patients and the overweight non-diabetic controls (2.3 ± 0.4 and 2.2 ± 0.5%, respectively). More than 40% of the greater mixed muscle lipid content was attributed to a higher proportion type I muscle fibres (62 ± 8% versus 38 ± 3 and 33 ± 7%, respectively), which contained 2.8 ± 0.3 fold more lipid than the type II fibres. The remaining difference was explained by a significantly greater IMCL content in the type I muscle fibres of the trained athletes. Differences in IMCL content between groups or fibre types were accounted for by differences in lipid droplet density, not lipid droplet size. IMCL distribution showed an exponential increase in lipid content from the central region towards the sarcolemma, which was similar between groups and fibre types.
**Conclusion:** IMCL contents can be substantially greater in trained endurance athletes compared to overweight and/or type 2 diabetes patients. As structural characteristics and intramyocellular distribution of lipid aggregates are shown to be similar between groups, we conclude that elevated IMCL deposits are unlikely to be directly responsible for inducing insulin resistance.

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**Skeletal muscle ceramide content is increased in lean nondiabetic, insulin resistant offspring of Type 2 diabetic subjects**

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**Background and aims:** Skeletal muscle is the important site of insulin action. 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. It is well-known that insulin resistance might be present also in normal weight humans, especially with positive family history of type 2 diabetes. Therefore, 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 nondiabetic offspring of type 2 diabetic subjects.
**Materials and methods:** The study group consisted of 18 lean (BMI<25 kg × $m^{-2}$) healthy male subjects, 9 offspring of type 2 diabetic patients and 9 individuals without family history of type 2 diabetes. 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:** Offspring of type 2 diabetic subjects were markedly more insulin resistant (p=0.019). We found significantly higher muscle ceramide content in the offspring group (p=0.015). Muscle ceramide was inversely related to insulin sensitivity (r =−0.56, p=0.015). The studied group did not differ in muscle sphingomyelin content, activities of sphingomyelinases and sphingosine level. Muscle sphinganine was slightly higher in the offspring group, but this difference was not statistically significant (p=0.14). Muscle sphinganine was also related to insulin sensitivity (r =−0.51, p=0.037).
**Conclusion:** Our data show that muscle ceramide accumulation could be an early abnormality in the development of insulin resistance in predisposed subjects. It remains to be elucidated whether elevated content of ceramide was due to increased rat of de novo synthesis, sphingomyelin hydrolysis or decreased ceramide degradation.
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## 551

**IMCL content in human skeletal muscle is correlated to local insulin-stimulated glycogen synthesis**

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**Background and aims:** Intramyocellular (IMCL) triglyceride stores are an accessible form of energy for skeletal muscle, and high IMCL levels might interfere with glucose metabolism through a decrease of glucose utilization. Indeed some studies have shown that IMCL content of the skeletal muscle, as measured with $^1$H MR spectroscopy, is negatively correlated with insulin sensitivity as measured by *whole body* glucose uptake. Glycogen synthesis rate, measured by $^{13}$C MR spectroscopy, reflects *local* insulin-stimulated glucose uptake in skeletal muscle. We hypothesized that the correlation between IMCL content and insulin action should be stronger within the same skeletal muscle.
**Materials and methods:** We studied 20 subjects of various age (range 18–65) and body-mass index (range 18–40), who were non-smoking, and had no family history of diabetes, hypertension, or any other systemic disease. $^1$H-MRS was used to measure IMCL content in both soleus and gastrocnemius muscle, and $^{13}$C MR spectroscopy in combination with an euglycemic hyperinsulinemic (60 mU/$m^2$/min) clamp ($^{13}$C-enriched glucose infusion) to measure insulin-stimulated glycogen synthesis rate in calf muscle.
**Results:** Glycogen synthesis rate ranged from 80–200 µmol/kg muscle/ min. Glycogen synthesis rate strongly correlated with the whole body glucose infusion rate (r = 0.69, p = 0.0031). Relative IMCL content in the tibialis muscle ranged from 0.002 to 0.032 (mean 0.0098 ± 0.002), in the soleus muscle from 0.005 to 0.050 (mean 0.0153 ± 0.002) and in the gastrocnemius muscle from 0.003 to 0.048 (mean 0.0138 ± 0.003). IMCL content was inversely correlated with the glycogen synthesis rate of the soleus muscle and gastrocnemius muscle (r = −0.56 and r = −0.57 resp. $P < 0.05$ for both). Glycogen synthesis rate and IMCL content of the tibialis anterior muscle were not significantly correlated (r = −0.24, p = 0.4). There was a weak, correlation between whole body glucose uptake and IMCL content for each muscle type. The soleus muscle demonstrated a borderline significant correlation with IMCL content (r = −0.43, $P = 0.07$), whereas gastrocnemius and tibialis were non-significant (r = −0.43, $P = 0.2$ and r = −0.18, $P = 0.5$ respectively).
**Conclusion:** We conclude that IMCL content of the soleus and gastrocnemius muscle is correlated to insulin-induced glycogen synthesis rate in the same muscle. This correlation is stronger than between IMCL content and whole body glucose uptake, suggesting a cause and effect relationship.
*Dutch Diabetes Foundation*

## 552

**Impaired glucose tolerance (IGT): does skeletal muscle fatty acid handling after a high fat meal improve after weight loss in IGT men?**

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**Background and aims:** Insulin resistant conditions are characterized by an impaired ability to regulate substrate oxidation during insulin mediated (i.e. postprandial) and catecholamine stimulated (i.e. exercise) conditions. Does metabolic flexibility of skeletal muscle substrate handling after a meal improve after weight loss in IGT men?
**Materials and methods:** Skeletal muscle substrate metabolism was studied in 8 IGT men during baseline and after a high fat mixed meal (60 energy% fat) for 4 hours by means of the forearm balance technique. Insulin sensitivity was measured with a hyperinsulinemic euglycemic clamp. The men followed an 8-week very low calorie diet. Measurements were done after at least 2 weeks of energy balance.

**Results:** The men lost 14.0 ± 4.3 kg of body weight, of which led to a reduction in body fat percentage from 33.4 ± 2.9% to 25.7 ± 3.7% ($p<0.001$). Insulin sensitivity improved after weight loss (WL), the M-value pre WL was 21.7 ± 10.0 and increased to 37.9 ± 11.6 mmol/min/kgFFM ($p<0.01$). Fasting levels of free fatty acids (FFA), triglycerides (TG) and insulin decreased significantly. The increment in postprandial insulin was lower after WL ($AUC_{insulin}$ pre WL = 39.0 ± 12.6 mU/L/min vs $AUC_{insulin}$ post WL = 23.0 ± 10.6 mU/L/min), whereas postprandial changes in free fatty acid concentrations were comparable to before WL. Changes were neither seen in skeletal muscle glycerol flux or free fatty acid flux after WL, despite increased insulin sensitivity, whereas glucose disposal tended to be higher (glucose $flux_{120\ min}$ = 0.44 ± 0.29 vs 0.22 ± 0.26 nmol/100 ml tissue/min, p = 0.09). Local respiratory quotient in skeletal muscle also increased more postprandially (pre WL ΔRQ=0.033 ± 0.050 and post WL ΔRQ = 0.051 ± 0.169) after WL compared to before weight loss, which was attributable to a trend in a higher increase in carbohydrate oxidation (p=0.07) and a tendency for a stronger suppression of fat oxidation after the high fat meal.
**Conclusion:** Weight loss improves insulin sensitivity in IGT men, resulting in a tendency towards an improved skeletal muscle glucose disposal, and a better capacity to suppress muscle lipid oxidation and to stimulate glucose oxidation during postprandial conditions.

# PS 37
# Lipid metabolism, mechanisms

## 553

**Subtype selectivity of LXR activating compounds corresponds to differential regulation of ABCA1 and SREBP-1c genes in cells**
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**Background and aims:** Liver X receptors LXRα (NR1H3) and LXRβ (NRH2) are members of the nuclear receptor superfamily of ligand-activated transcription factors. The natural ligands are thought to be oxidized derivatives of cholesterol such as 22(R)-hydroxycholesterol, 24(S),25-epoxycholesterol and 27-hydroxycholesterol. Among the LXR target genes are several of those involved in cholesterol efflux from peripheral tissues such as the ATP-binding-cassette transporters ABCA1, ABCG1 and apolipoprotein E. Activation of these genes by LXR agonists may have antiatherogenic effects by increasing cholesterol efflux from vascular foam cells. In addition ABCA1 is necessary to generate mature high density lipoprotein particles (HDL), and ABCA1 activity is regarded as a major determinant of plasma HDL. Thus activation of LXR may lead to an increase in HDL-C and amplified reverse cholesterol transport. The drawback however has been the effect of LXR activating compounds on lipogenic genes like SREBP-1 c, FAS or SCD1. We used LXRβ selective ligands to test the hypothesis that the lipogenic effect is mediated mainly by the LXRα subtype.
**Materials and methods:** Recombinant ligand binding domains of LXRα and LXRβ were purified from bacterial extracts. The activation of the nuclear receptors was measured by the AlphaScreen technology. The effect on gene expression was studied by quantitative RT-PCR with a LightCycler Instrument.
**Results:** We have identified compounds with agonistic activity on LXRβ in vitro whereas the activity on LXRα is very low. However these compounds inhibit the activation of LXRα by the subtype unselective LXR activator T0901317. In contrast, on LXRβ our compounds and T0901317 exhibit additive agonistic activity. In THP-1 and Phi133 cells the compounds increase the expression of ABCA1 whereas the mRNA of SREBP-1 c is reduced in these cell lines.
**Conclusion:** Selective activation of LXRβ is sufficient to increase ABCA1 expression in vitro but does not lead to the substantial upregulation of SREBP-1 c seen with unselective LXR activators. Thus LXRβ selective compounds may be useful for the treatment of dyslipidemia and cardiovascular disease.

## 554

**The increased production of endogenous fatty acids impairs the utilization of exogenous palmitate in vitro in insulin resistant hereditary hypertriglyceridemic rats**
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**Background and aims:** Glucose and fatty acids are main energy substrates. The preference of their utilization and their metabolic fate is different under normal conditions and in insulin resistant state. The aim of our study was to determine how these two substrates in physiological concentrations are utilized in vitro in skeletal muscle and adipose tissue in their simultaneous presence in non-obese hereditary hypertriglyceridemic (HHTg) rats, genetically fixed model of IR.
**Materials and methods:**C and HHTg (weight 300 ± 20 g) rats were fed for two weeks high sucrose (HS, 70% of calories as sucrose) diet. The distal parts of epididymal adipose tissue (EAT) and musculus soleus were incubated 120 min in Krebs-Ringer buffer with 5 mmol/l glucose and 0,5 mmol/l palmitate with either $^{14}$C-glucose or $^{14}$C-palmitate. The incorporation of labeled substrate into triglycerides (Tg), glycogen and $CO_2$ in musculus soleus and into Tg in EAT during in vitro incubation was determined. The incorporation of glucose into Tg was considered to be an indicator of FA esterification. The in vitro lipolysis was assessed as glycerol release.
**Results:** HS feeding led to significantly higher postprandial triglyceridemia (5,1 ± 0,3 vs 2,1 ± 0,4 mmol/l) and higher concentration of fasting serum NEFA (1,3 ± 0,1 vs 1,0 ± 0,05 mmol/l) in HHTg group compared with controls. The data obtained in vitro in skeletal muscle and EAT are given in the

table. In soleus muscle the distribution of incorporated glucose among three main metabolic pathways was entirely different in HHTg and control rats. In controls, 68% of glucose was incorporated into glycogen, the rest of glucose was nearly equally divided between oxidation and incorporation into Tg. In HHTg, the glycogenesis was attenuated and $CO_2$ production was potentiated. The incorporation of glucose into muscle Tg was higher in HHTg compared to controls but it was not accompanied by higher incorporation of exogenous palmitate. The oxidation of palmitate was higher in control group. In EAT the incorporation of glucose into Tg was higher in HHTg than in C rats. In contrast, the incorporation of exogenous palmitate into Tg was in HHTg group lower. The unstimulated in vitro lipolysis was higher in HHTg rats compared with controls (3.2 ± 0.3 vs 2.5 ± 0.16 mmol/g w.wt.).

**Conclusion:** We conclude, that exogenous glucose is more utilized for esterification of fatty acids generated by increased hydrolysis of endogenous Tg in HHTg compared to control rats both in adipose tissue and skeletal muscle. This implicates higher turnover of endogenous Tg in HHTg. The decreased utilization of exogenous fatty acids (both oxidation and esterification) in IR animals may contribute to the pathological increase of their concentration in serum.

| incorporation of | | $^{14}$C-glucose control nmol/g w.wt. | % | HHTg nmol/g w.wt. | % | $^{14}$C-palmitate control nmol/g w.wt. | HHTg nmol/g w.wt. |
|---|---|---|---|---|---|---|---|
| | into $CO_2$ | 210 ± 20 | 19 | 320 ± 10$^{+}$ | 34 | 76 ± 7 | 49 ± 3$^{++}$ |
| soleus | into Tg | 150 ± 10 | 13 | 250 ± 20$^{+++}$ | 27 | 250 ± 20 | 230 ± 10 |
| | into glycogen | 1460 ± 40 | | 1860 ± 130$^{+}$ | | 1020 ± 40 | 810 ± 55$^{+}$ |
| EAT | into Tg | 1460 ± 40 | | 1860 ± 130$^{+}$ | | 1020 ± 40 | 810 ± 55$^{+}$ |

+ significant difference HHTg vs C; + $p < 0.05$; +++$p < 0.001$

*Supported by: IGA MH CR.*

## 555

**Overexpression of Acyl CoA Synthetase 1 in liver promotes triglyceride deposition by partitioning intracellular fatty acid into storage pathways: *in vivo* studies in rodents**

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**Background and aims:** Excess fat deposition in non-adipose tissues is strongly associated with metabolic disease including insulin resistance, but underlying mechanisms leading to lipid accumulation are poorly understood. Activation of intracellular fatty acid (FA) to long chain acyl CoA by Acyl CoA Synthetase (ACS) is an obligatory first step towards either FA oxidation or the production of triglyceride (TG). It has been proposed that discrete pools of acyl CoA produced by different isoforms of ACS (ACS1-5) may have particular metabolic fates. Here we have tested *in vivo* the hypothesis that ACS1 preferentially channels FA towards TG formation.

**Materials and methods:** Adenoviral-mediated gene transfer was used to overexpress ACS1 in the livers of male C57BL/6J mice and Wistar rats. The active adenovirus (AdACS1) was constructed using the AdEasy system and contained the rat ACS1 cDNA and GFP cDNA under control of cytomegalovirus promoters. An adenovirus containing only GFP (AdGFP) was used as a control. Adenovirus was administered either by injection into a tail-vein (mice) or jugular cannula (rats) resulting in liver-specific infection. The major experimental endpoint in mice was the timecourse of hepatic accumulation of lipids. In rats the intracellular partitioning of fatty-acids (FA) between oxidation and storage was determined directly *in vivo* by our established tracer methodology employing simultaneous administration of the fatty-acid analog R-Bromopalmitate ($^{3}$H-R-BrP) and palmitate ($^{14}$C-P). Because the analog $^{3}$H-R-BrP is not oxidised, tissue entrapment of $^{3}$H label provides an index of total FA uptake. In contrast the oxidative products of the authentic FA $^{14}$C-P are rapidly exported; the $^{14}$C label that remains is used to quantify the conversion of plasma FA to intracellular storage products.

**Results:** In mice, expression in liver peaked at four days post-infection when ACS1 mRNA and protein were increased >5-fold over controls. ACS1 overexpression caused a 2-fold increase in TG deposition in liver (39 ± 4 compared to 20 ± 2 μmol/g in controls, $p<0.005$). Rats were studied under anaesthesia four days after infection. Liver ACS1 protein expression was double ($p<0.02$) that of controls, but this did not alter total hepatic FA clearance (AdACS1: 89 ± 12, AdGFP: 88 ± 3 ml.min$^{-1}$100g$^{-1}$; $p=0.9$). However the fraction of FA that was directed to storage increased from 56 ± 4% to 78 ± 4% ($p<0.02$). Overall there was a strong correlation ($R^2=0.79$, $P=0.003$) between this partitioning ratio and ACS1 protein expression.

**Conclusion:** Adenoviral-mediated gene transfer was used here to overexpress ACS1 in the livers of rodents. In mice, ACS1 overexpression promoted triglyceride deposition in the liver. ACS1 overexpression in rats increased the fraction of fatty acid that was directed towards synthesis of storage products, without increasing the overall efficiency of the liver to clear circulating fatty acids. Together, these experiments strongly support an important role for ACS1 in controlling the partitioning of intracellular fatty acid between triglyceride formation and β-oxidation.

## 556

**Suppression of hyperinsulinemia by diazoxide attenuates hepatic lipogenesis in Zucker diabetic fatty rats**

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**Background and aims:** Chronic attenuation of hyperinsulinemia by diazoxide (DZ), an inhibitor of glucose-mediated insulin secretion, in pre-diabetic Zucker diabetic fatty (ZDF) rats, an animal model of type 2 diabetes and leptin resistance, decreased rate of weight gain, prevented diabetes and improved lipid profile. Since hepatic lipogenic enzyme gene expression is regulated by the opposing effects of insulin and leptin, we studied the effect of chronic insulin suppression on key insulin-sensitive genes regulating hepatic lipogenesis.

**Materials and methods:** DZ (150 mg/kg per day) or vehicle [pair-fed (PF) and control (C)] was administered to 6-week-old pre-diabetic obese male ZDF and ZDF lean (ZL) rats for 8 weeks.

**Results:** DZ increased fasting plasma insulin ($p<0.001$), but decreased fasting glucose ($p<0.0001$) in ZDF rats without a significant change in DZ lean animals. DZ lowered plasma leptin ($p<0.001$), free fatty acids ($p<0.02$), cholesterol ($p<0.02$) and triglycerides ($p<0.001$) levels, but increased adiponectin levels ($p<0.02$) in ZDF rats without a significant effect in DZ rats. Insulin receptor substrate-1 protein expression was lower in ZDF compared to ZL rats ($p<0.005$) and was enhanced in DZ-ZDF ($p<0.02$) and ZL ($p<0.03$) rats. While total protein kinase B (Akt) levels were similar in both strains, ZDF had lower phosphorylated Akt (p-Akt) levels than ZL rats ($p<0.01$). DZ-treated ZDF and ZL rats had higher levels of p-Akt than their controls ($p<0.03$). This was accompanied by reduction in expression of sterol regulatory element-binding protein-1 c, ($p<0.0001$), fatty acid synthase ($p<0.002$), acyl CoA carboxylase ($p<0.001$), hormone-sensitive lipase ($p<0.005$) and peroxisome proliferator agonist receptor-γ (($p<0.02$) in ZDF rats. However, DZ treatment did not affect the expressions of acyl CoA oxidase, peroxisome proliferator receptor-α, and carnitine palmitoyl transferase-1. DZ treatment caused significant reduction in hepatic triglycerides ($p<0.001$), long chain acyl-CoA ($p<0.001$) and cholesterol ($p<0.01$) contents in ZDF rats without any effect in ZL animals.

**Conclusion:** Chronic suppression of hyperinsulinemia prevented the development of diabetes in ZDF rats. This was accompanied by enhanced hepatic insulin sensitivity and decreased expression of key genes regulating hepatic lipogenesis without altering genes regulating lipid oxidation. These results suggest that attenuation of hyperinsulinemic state by DZ enhances metabolic efficiency of insulin and is therapeutically beneficial.

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## 557

**Lack of association between serum paraoxonase 1 activity and circulating oxidized low-density lipoprotein in Type 2 diabetic patients**

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**Background and aims:** Among mechanisms that may explain the antiatherogenic properties of high density lipoprotein (HDL), paraoxonase (PON1), an esterase linked to the apolipoprotein A1, containing HDL fraction, has raised particular interest. PON1 inhibits the oxidation of low-density lipoprotein (LDL) in vitro, preventing accumulation of lipoperoxides and destroying oxidized phospholipids in modified LDLs. The aim of the present study was to determine serum PON1 activity and the distribution of two PON1 gene polymorphisms (L/M, Leu55Met and Q/R, Gln192Arg) in patients with type 2 diabetes and to relate them to levels of circulating oxidized LDL (oxLDL).

**Materials and methods:** Paraoxonase activity and genotypes were therefore investigated in 317 caucasian people with type 2 diabetes and 106 healthy subjects. Serum PON1 activity was measured spectrophotometrically using paraoxon as a synthetic substrate. RFLP was used to screen for PON1 Leu55Met (NlaIII enzyme) and Gln192Arg (AlwI enzyme, New England

Biolabs) polymorphisms. Serum level of total oxLDL particles was measured by a sandwich ELISA assay (Mercodia AB, Uppsala, Sweden).
**Results:** PON1 activity did not differ in diabetic and controls (66 ± 41 vs 58 ± 44 nmol/min/mL, p=0.09). PON1 activity does not change with age or diabetes duration and is not related to BMI, while an inverse regression can be described with HbA1 c (r =–0.152, p=0.018), so that subject with PON activity in the two higher quartiles have HbA1 c significantly lower than diabetics in the first PON1 activity quartile (p=0.03). In type 2 diabetics the L55 allele frequency was 0.57 (M55: 0.43) and that of the Q192 allele was 0.66 (R192: 0.34), both superimposible to those observed in controls. As far the PON1-192 polymorphism, the QR genotype was the most common (50.8%) followed by the QQ (41.0%) and the rare RR (8.2%); for PON1-55, the LM genotype was the most common (67.6%) followed by LL (23.4%) and MM (9.0%). The distribution of PON1 activity by genotype were the same in both population; for PON1-55, LL>LM>MM and for PON1-192, RR>RQ>QQ. The two polymorphisms exert addictive effects on PON1 activity so that the lowest values were observed in QQ + MM while the highest levels for RR + LL (p=0.0008). By a logistic regression analysis, PON1-192 polymorphism (p<0.0001), PON1-55 polymorphism (p=0.03), ApoA1 (p<0.05) and HbA1 c (p<0.05) independently contribute to PON1 activity variability. OxLDL levels did not significantly differ in controls (49.0 ± 11.1U/L) and type 2 diabetics (55.6 ± 25.3U/L, p=0.16). PON1 activity does not correlate with any lipid parameter other than ApoA1 (r=0.17, p=0.0029). PON1 activity was not related to the levels of oxLDL (r=0.05, p=0.35), and no differences in oxLDL concentrations were observed by quartiles of PON1 activity. The two PON1 polymorphisms did not affect the lipid profile; furthermore, neither PON1-192, nor PON1-55 or their combination affect serum oxLDL levels. Finally, the relationship between PON1 activity and the levels of oxLDL was not affected by the PON1 genotypes.
**Conclusion:** Our data suggest that, in type 2 diabetic patients variations in PON1 activity and PON1 gene polymorphisms (that regulate PON1 activity) does not affect the degree of circulating LDL oxidation.

# PS 38
# Lipids and lipoproteins in diabetes

## 558

**Simvastatin increases HDL cholesterol and decreases plasma cholesteryl ester transfer in Type 1 diabetes mellitus**
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**Background and aims:** Statin treatment may increase HDL cholesterol by affecting plasma cholesteryl ester transfer in type 1 diabetes mellitus. We evaluated the effect of various doses of simvastatin on plasma (apo) lipoproteins, plasma lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein and phospholipid transfer protein (PLTP) activities and on plasma cholesterol esterification (EST) and cholesteryl ester transfer (CET) in type 1 diabetes mellitus.
**Material and methods:** Fourteen non-smoking male type 1 diabetes mellitus patients without microalbuminuria (age 45 ± 14 years, BMI 25.9 ± 3.0 kg/m$^2$) with a baseline plasma total cholesterol > 5.0 mmol/l (5.60 ± 1.03 mmol/l) were studied in a randomized double blind design. Participants received simvastatin 10 mg, 20 mg, 40 mg daily and placebo with each period lasting 6 weeks. Fasting plasma (apo)lipoproteins, plasma LCAT, CETP, PLTP activities (assayed by isotope methods that measure the active amounts of these proteins) as well as plasma EST and CET were measured at the end of each treatment period.
**Results:** Weight and $HbA_{1c}$ remained unaltered during simvastatin treatment. Simvastatin therapy decreased plasma total cholesterol, VLDL+LDL cholesterol, triglycerides and apo B levels compared to placebo ($p < 0.05$ to $p < 0.01$). The largest decreases in plasma total cholesterol, VLDL+LDL cholesterol and apo B levels were seen in response to the highest simvastatin dose. HDL cholesterol increased ($p < 0.05$) during all simvastatin treatment periods by 3 to 6% without a dose-related effect. Plasma apo A-I increased significantly after simvastatin 20 mg. Plasma CET, CETP activity and EST decreased after simvastatin treatment ($p < 0.05$ to $p < 0.01$). No significant changes in plasma LCAT activity and PLTP activity were observed in response to simvastatin. The increase in HDL was negative correlated with the decrease in plasma CET after simvastatin 40 mg daily ($R_S$ = –0.66, $p < 0.02$). In turn, the decrease in plasma CET after simvastatin 40 mg daily was correlated with the decrease in VLDL+LDL cholesterol ($R_S = 0.90$, $p = 0.001$), the decrease in plasma triglycerides ($R_S = 0.69$, $p < 0.02$) and the decrease in plasma CETP activity ($R_S = 0.58$, $p < 0.05$) but not with the changes in PLTP activity ($R_S = 0.27$, N.S.).
**Conclusion:** Simvastatin therapy increases HDL cholesterol in type 1 diabetes mellitus. It is likely that a decrease in plasma cholesteryl ester transfer, which is due a drop in apo B-containing lipoproteins as well as in plasma CETP activity, provides a mechanism to explain the rise in HDL cholesterol in response to this treatment.

## 559

**Ezetimibe added to statin therapy reduces LDL-C and improves goal attainment in patients with diabetes, metabolic syndrome, or metabolic dyslipidemia**
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**Background and aims:** Patients with diabetes (DM) and patients with metabolic syndrome (MS) but without frank diabetes often do not reach NCEP ATP III target goals for LDL-C despite treatment with statins. These patients frequently exhibit a dyslipidemia characterized by low levels of HDL-C, elevated levels of triglyceride (TG), and TG-rich lipoproteins and these lipoprotein abnormalities independently impart an increased risk for CHD.
**Materials and methods:** In a randomized clinical trial, we examined the additional LDL-C lowering efficacy and safety of ezetimibe 10 mg (EZE) compared with placebo added to a stable dose of any statin in 3030 patients (299 US sites) with LDL-C levels exceeding NCEP ATP III goals. Patients using marketed statins (approx. 40% atorvastatin, 29% simvastatin, 22%

pravastatin, and 10% other) were randomized 2:1 to EZE and placebo. We examined percent changes from baseline in LDL-C and other lipids and the percent of patients reaching NCEP ATP III LDL-C goal after 6 weeks of treatment.
**Results:** More than one-third (38.5%) of the patients had DM and 26.9% had MS without frank diabetes (≥3 NCEP ATP III criteria), which were predefined as categories for subanalysis. We also analyzed patients with metabolic dyslipidemia (MD) (24.6%), characterized by both low HDL-C and elevated TG (HDL-C< 40 mg/dL [men] or <50 mg/dL [women]; TG > 150 mg/dL) and at least 1 other characteristic of metabolic syndrome, which could include impaired glucose tolerance or frank diabetes. In the patients with DM, mean baseline HbA1C was similar in both treatment groups.
In each of these subgroups, non-HDL-C, apolipoprotein B, HDL-C, TG, and Total-C improved significantly ($p<0.05$ between-treatment difference within subgroup) in patients treated with EZE, relative to placebo, plus ongoing statin therapy. EZE was well tolerated with a safety profile comparable to statin alone.
**Conclusion:** The addition of EZE to ongoing statin therapy in patients who have not attained their NCEP ATP III LDL-C goal and who have DM, MS without frank diabetes, or MD resulted in significant additional reductions in LDL-C and improvements in other lipid parameters, and significant improvements in LDL-C goal attainment.

Treatment differences for LDL-C lowering and goal attainment for patients not at goal at baseline

| Subgroup Treatment Group | N | Baseline (mean) | Percent change from baseline LDL-C (mean [SE])† | Between-treatment differences (mean [95% CI])† | Number of patients not at goal at base-line | Percent patients reaching LDL-C goal‡ |
|---|---|---|---|---|---|---|
| **Diabetes** | | | | | | |
| Placebo + statin | 387 | 121.6 | -3.0 (1.1) | -24.8 (-27.0, -22.6)* | 317 | 21.1 |
| EZE10 mg + statin | 739 | 121.5 | -27.8 (0.9) | ---- | 606 | 71.1* |
| **Metabolic Syndrome w/o DM** | | | | | | |
| Placebo + statin | 245 | 132.2 | -2.8 (1.2) | -21.4 (-24.1, -18.7)* | 43 | 21.9 |
| EZE10 mg + statin | 528 | 134.0 | -24.3 (0.9) | ---- | 289 | 67.1* |
| **Metabolic Dyslipidemia** | | | | | | |
| Placebo + statin | 228 | 129.5 | -2.9 (1.3) | -22.7 (-25.5, -19.9)* | 185 | 25.4 |
| EZE10 mg + statin | 489 | 130.7 | -25.6 (1.0) | ---- | 407 | 65.1* |

*p<0.001 (between-treatment difference); † Least square means; ‡ For DM patients, LDL-C goal <100 mg/dL; for MS and MD patients, goal was determined by NCEP risk category

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## 560

### Influence of simvastatin on serum levels of apolipoproteins and leptin in patients with Type 2 diabetes mellitus

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**Background and aims:** There are convincing evidences of beneficial role of correction of dyslipidemia with statins in reduction of cardiovascular morbidity and mortality in patients with diabetes. However, not all mechanisms of positive action of statins are fully understood. Therefore, the aim of this study was to investigate the influence of treatment with simvastatin on serum levels of apolipoproteins A and B and leptin in patients with type 2 diabetes.
**Materials and methods:** We studied 20 patients with type diabetes mellitus (age 59.1±1.69 years old, BMI – 31.9±1.07 kg/m$^2$, diabetes duration – 5.6±1.25 years, data are presented as mean±SEM) who were treated simvastatin 20 mg once daily for 6 weeks. Serum cholesterol, LDL, triglycerides, apoipoprotein A and B and leptin levels were measured before and after treatment with simvastatin. Statistical analysis was performed by Student's paired test.
**Results:** Treatment with simvastatin resulted in significant reduction of total cholesterol ($6.97 \pm 0.22$ vs. $4.97 \pm 0.20$ mmol/L, $p < 0.05$), LDL ($4.62 \pm 0.19$ vs. $2.92 \pm 0.18$ mmol/L, $p<0.05$), triglycerides serum levels ($2.79 \pm 0.20$ vs. $1.79 \pm 0.13$ mmol/L, $p<0.05$), and an increase of HDL ($1.08 \pm 0.06$ vs. $1.24 \pm 0.05$ mmol/L, $p<0.05$ before and after treatment, respectively). Moreover, treatment with simvastatin led to significant increase of serum apoliporotein A levels – $144.08 \pm 5.82$ vs. $158.98 \pm 4.80$, $p<0.05$ and decrease of apoliporotein B levels – $148.88 \pm 7.99$ vs. $120.62 \pm 8.02$, $p < 0.05$ and apoB/apoA lipoproteins ratio – $1.06 \pm 0.06$ vs. $0.77 \pm 0.06$, $p<0.05$ before and after treatment, respectively. It was trend toward decrease of serum leptin levels as the result of simvastatin use – $7.74 \pm 1.52$ vs. $6.61 \pm 1.18$, $p=0.07$.
**Conclusion:** The treatment with simvastatin resulted in improvement of dyslipidemia, positive changes of serum apolipoproteins levels with the trend toward reduction of serum leptin level. These changes could contribute to the positive effect of simvastatin on the reduction of cardiovascular risk in patients with type 2 diabetes mellitus.

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### Pattern recognition discrimination of Type 2 diabetic patients from healthy controls based on their serum fatty acid profiles

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**Background and aims:** Fatty acids are known to be important biomedical indicators of the abnormal lipid metabolism in diabetes mellitus and extensive studies have been undertaken to investigate the changes of fatty acid profiles in type 2 diabetic patients. The aim of this study was to find out if it is possible to use the serum fatty acid profiles to classify diabetics and controls and to identify characteristic features which are relevant for this classification.
**Materials and methods:** 51 type 2 diabetic patients and 50 healthy controls were included in the study. Fatty acid profiles (21 individual fatty acids) of five serum lipid fractions (total of 105 fatty acids/subject) were determined in diabetic patients and healthy controls by capillary gas chromatography. Principal component analysis (PCA) and univariate statistical analysis were carried out for each lipid fraction.
**Results:** First results indicated that the fractions of cholesterol esters, free fatty acids and phospholipids provided strong classification capability between patient and control groups, whereas the triglyceride fraction did not. A mixed univariate/multivariate feature selection strategy was applied to each fraction of fatty acids. The subsets of fatty acids of all fractions were combined to generate a combination data set and again the feature selection was carried out on the combination data set to select optimum features for final classification. Linear discriminant analysis (LDA) and artificial neural networks (ANNs) were applied for differentation of diabetic patients from controls based on the selected features. Both LDA and ANNs yielded satisfactory classification results, while the ANNs' performance was relatively superior. The combination of fatty acids of all fractions provided better classification results than individual fraction of fatty acids. ANN classification results for the combination of fatty acids from all lipid fractions showed a recognition rate of 95.5%, a prediction rate of 89.7%, sensitivity of 88.2% and specificity of 90.9%.
**Conclusion:** In this study, it was suggested that serum fatty acid profiles determined by capillary gas chromatography combined with pattern recognition analysis of the data may provide an effective approach to the discrimination of diabetic patients from healthy controls. It was shown that using univariate and multivariate strategy, it is possible to find the optimum combination of serum fatty acids to provide better performance.

## 562

### Human plasma phospholipid transfer protein activity is decreased by acute hyperglycaemia: studies without and with hyperinsulinaemia in type 1 diabetes mellitus

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**Background and aims:** Little is known about the regulation of phospholipid transfer protein (PLTP), that plays a key role in lipoprotein metabolism. PLTP secretion may be upregulated by glucose *in vitro*, whereas plasma PLTP activity is decreased by exogenous hyperinsulinaemia and glucose-induced hyperinsulinaemia *in vivo*. In the present study, we evalu-

ated the separate effects of hyperglycaemia and hyperinsulinaemia in C-peptide negative type 1 diabetic patients.
**Material and methods:** The protocol was carried out in 16 patients (8 females). In each patient, plasma PLTP mass and activity (measured by enzyme-linked immuno-sorbent assay and liposome-high density lipoprotein system, respectively) as well as plasma cholesteryl ester transfer protein (CETP) activity, lipids and apolipoprotein levels were determined at the end of 4 different glucose clamps, each lasting 210 min: standard insulin (30 mU $kg^{-1}$ $h^{-1}$) and standard glucose (glucose 5.0 mmol $L^{-1}$) (SI-SG), standard insulin and high glucose (glucose 12 mmol $L^{-1}$) (SI-HG), high insulin (150 mU $kg^{-1}$ $h^{-1}$) and standard glucose (HI-SG), and high insulin and high glucose (HI-HG).
**Results:** Plasma lipids and (apo)lipoproteins, measured at the end of the SI-HG, HI-SG and HI-HG clamps, were not significantly different compared to the levels obtained at the end of the SI-SG clamp. Median plasma PLTP mass and activity at the end of the SI-SG clamp were 12.8 mg $L^{-1}$ and 13.2 µmol $mL^{-1}$ $h^{-1}$, respectively. Median plasma PLTP mass was decreased 9.1% at the end of the HI-HG clamp ($P < 0.05$), whereas the changes at the end of the SI-HG and HI-SG clamps were not significant. Median plasma PLTP activity decreased by 5.7%, 4.6%, and 8.6% at the end of the SI-HG, HI-SG and HI-HG clamps, respectively (all $P < 0.05$). Median plasma CETP activity was 177 nmol $mL^{-1}$ $h^{-1}$ at the end of the SI-SG clamp, and decreased by 4.9% ($P < 0.05$) and by 8.3% ($P < 0.05$) at the end of the HI-SG and the HI-HG clamps, respectively. Plasma CETP activity did not change significantly at the end of the SI-HG clamp.
**Conclusions:** The present study demonstrates that plasma PLTP activity is independently decreased by acute hyperglycaemia and hyperinsulinaemia in humans *in vivo*. These data do not support a direct role of short-term hyperglycaemia in upregulating plasma PLTP levels.

# PS 39
# Insulin secretion in animals

## 563

**Early versus late insulin secretion as determinants for glucose tolerance in mice**
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**Background and aims:** Correct estimation of insulin secretion in vivo requires quantification in relation to insulin sensitivity, since these two variables are mutually dependent displaying an inverse non-linear relation, best explained by a hyperbolic function. This provides the disposition index obtained by multiplying insulin secretion times insulin sensitivity. However, in view of the dynamic pattern of insulin secretion with an early and a late phase, it is not yet known which measure of insulin secretion after an intravenous glucose challenge that should be used when relating to insulin sensitivity. Such knowledge would be important not only for pathophysiological studies, but also for studies when developing new treatment for diabetes. The aim of this study, therefore, was to evaluate the early versus total insulin secretion in relation to glucose tolerance and insulin sensitivity during an intravenous glucose tolerance test (IVGTT) with multiple sampling in mice.
**Materials and methods:** Anesthetized female C57BL/6J mice (n=203) were injected intravenously with glucose (1g/kg). Blood samples for determination of insulin and glucose were taken at t=0, 1, 5, 10, 20, 30 and 50 min. The early insulin secretion was quantified as mean of 1 and 5 min insulin levels ($AIR_G$) and as suprabasal $AIR_G$ ($\Delta AIR_G$), and the late insulin secretion was calculated as the area under the 10–50 min insulin curve (AUC). Insulin sensitivity was estimated as the insulin sensitivity index ($S_I$) using minimal model analysis of the insulin and glucose data during the IVGTT. Glucose disposal was quantified as the glucose elimination constant ($K_G$) for the 5–20 min interval as percent per minute. Linear and non/linear correlations were performed between insulin secretion measures versus $K_G$ and $S_I$.
**Results:** The intravenous administration of glucose resulted in a sharp peak of insulin at min 1, followed by a nadir at min 5 and a subsequent ascending late phase. Also glucose levels peaked at min 1 which was followed by a gradual return to baseline levels. $K_G$ was $2.40 \pm 0.06$%/min. $AIR_G$ was $1126 \pm 57$ pmol/l, $\Delta AIR_G$ was $830 \pm 41$ pmol/l and AUC was $26.6 \pm 1.03$ nmol/l in 50 min. $S_I$ was $0.79 \pm 0.03$ $10^{-4}$ $min^{-1}$/(pmol/l). Early insulin secretion showed higher correlation with $K_G$ ($r=0.38$ for $AIR_G$ and $r=0.45$ for $\Delta AIR_G$, both $P<0.001$) than late insulin secretion ($r=0.25$, $P=0.039$ for AUC). The estimates of the early insulin secretion related to $S_I$ in an inverse non-linear, hyperbolic, manner, being $\Delta AIR_G$ the most significant ($r=0.90$, $P<0.0001$). In contrast, AUC did not relate significantly to $S_I$.
**Conclusion:** We conclude that 1) the early insulin response shows a better correlation with the $K_G$ than the late insulin secretion during an IVGTT, 2) estimates of early but not late insulin secretion inversely relate to $S_I$ in a hyperbolic manner, and 3) the suprabasal early insulin response ($\Delta AIR_G$) shows better correlation to $K_G$ and $S_I$ than $AIR_G$. Therefore, relating insulin secretion to insulin sensitivity and calculating disposition index during an IVGTT in mice should preferably include the suprabasal early insulin response to glucose ($\Delta AIR_G$).

## 564

**Effects of GLP-1 and GIP on insulin secretion obtained with C-peptide deconvolution in mice**
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**Background and aims:** In model experiments in mice, insulin secretion is usually estimated from peripheral measurements of plasma insulin following challenges with glucose or other secretagogues. There are, however, problems with this approach because of the delay between secretion and change in circulating levels and the large proportion of insulin that is degraded in the liver. This problem may be circumvented by calculating insulin secretion at β-cell level using C-peptide concentrations. In several species, this analysis has been performed using C-peptide deconvolution. Aims of this study were to apply this analysis to mice, to exploit more sophisticated assessment by segregating secretion into specific components and to examine the effect of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) on insulin secretion as assessed by C-peptide deconvolution.

**Materials and methods:** Anesthetized female NMRI mice were injected iv with human C-peptide (3 nmol/kg) in combination with ip diazoxide (25 mg/kg; n=61), or (n=165) iv with glucose alone (1 g/kg) or glucose plus GLP-1 (2.8 nmol/kg) or GIP (2.8 nmol/kg). Blood samples for measurements of C-peptide were taken at 0, 1, 5, 10, 20, 30 and 50 min after injection. C-peptide kinetics was evaluated with a two-compartment model using non-linear mixed effects population analysis. Using parameters of C-peptide kinetics, prehepatic insulin secretion was reconstructed by deconvolution, and segregated in promptly secreted insulin (<1 min), early phase insulin secretion (≈1–10 min) and late phase insulin secretion (≈10–25 min).
**Results:** C-peptide kinetics analysis revealed fractional C-peptide clearance of 0.079 ± 0.005 (SEM) $min^{-1}$, corresponding to a half-life in the system of 8.8 ± 0.5 min. C-peptide distribution volume was 11.4 ± 0.4 ml and C-peptide clearance was 0.92 ± 0.04 ml/min. The Table shows the amount of insulin secreted above baseline (that was 16.2 ± 0.3 pmol/min) during the prompt (<1 min) and early phases (≈1–10 min) in response to glucose alone, to glucose+GLP-1 and to glucose+GIP. It is seen that the largest amount of insulin in response to glucose is secreted promptly (within 1 min). GLP-1 and GIP markedly augmented insulin secretion during both phases. After GLP-1 and GIP, the early phase (≈1–10 min) becomes more important than the prompt component. In contrast, there was no significant suprabasal insulin secretion during the late phase (10–50 min) in any group.

| | Glucose | Glucose+GLP-1 | Glucose+GIP |
|---|---|---|---|
| Prompt phase | 9.1 ± 1.4 | 31.2 ± 2.7* | 21.9 ± 4.0* |
| Early phase | 3.0 ± 2.1 | 54.9 ± 4.0* | 23.3 ± 2.7* |

Insulin secreted (pmol) during two segregated components. * P<0.001 vs glucose alone.
**Conclusion:** We conclude 1) that C-peptide deconvolution for estimating prehepatic insulin secretion is possible to be performed in mice, 2) that sophisticated analysis reveals segregated components of insulin secretion, 3) that glucose-stimulated prehepatic insulin secretion is markedly augmented by GLP-1 and GIP, and 4) that after glucose administration, insulin secretion is largely occurring as a prompt release during the first minute, while GLP-1 and GIP delay the predominant phase.

## 565

**Abnormalities of neuronal NO synthase activity and expression are implicated in the hypersecretion of insulin in obese Zucker fa/fa rats.**
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**Background and aims:** Zucker fa/fa rats are hyperphagic, obese, insulino-resistant and hyperinsulinic animals, due to a mutation in the leptin receptor. The hypersecretion of insulin is persistent even if islets are maintained in culture for a long period, suggesting a constitutive defect of pancreatic β-cell. Our previous studies have shown that an isoform of neuronal NO synthase (nNOS) is expressed and controls the kinetics and the amplitude of insulin secretion in rat pancreatic β-cells. Therefore, our aim was to search for abnormalities of nNOS that could at least partly account for Zucker fa/fa β-cell dysfunction.
**Materials and methods:** nNOS functional activity has been evaluated in the perfused rat pancreas by studying 1) the effects of its substrate arginine in the presence of 2.8 mM glucose and 2) those of two pharmacological inhibitors of nNOS, Nω-nitro-L-arginine methyl ester (L-NAME) and 7-nitroindazole (7-NI) during a glucose challenge (4.2 to 11 mM). nNOS expression was studied by Western blot using a monoclonal anti-nNOS antibody and a monoclonal anti-α-tubulin for normalization of the extracts. Dimerization of nNOS was analyzed by low temperature SDS-PAGE without boiling the samples, followed by a Western blot.
**Results:** In the presence of L-NAME (5 mM), the biphasic exaggerated insulin response to glucose that occurs in fa/fa rats was converted into a monophasic reduced one (653 ± 162 ng versus 1545 ± 252 ng × 20 min, $P < 0.01$). In fa/+ rats, the inhibitor also induced a monophasic pattern, but the magnitude of the response to glucose remained unchanged (485 ± 94 versus 591 ± 66 ng × 20 min). When 7-NI (100 μM) was used as an inhibitor during high glucose, a drastic increase in insulin response occurred in both fa/+ and fa/fa rats (3677 ± 757 versus 1545 ± 252 ng × 20 min in fa/fa rats, $P < 0.01$, and 1649 ± 154 versus 591 ± 66 ng × 20 min in fa/+ rats, $P < 0.01$). Arginine (5 mM) in the presence of 2.8 mM glucose induced a moderate monophasic response that is slightly higher in the fa/fa rats as compared to lean animals (NS). The addition of L-NAME (5 mM) strongly increased the two responses that both displayed a biphasic secretory pattern. To investigate the reason for the discrepancies observed, we studied nNOS expression by Western blot and found a two-fold increase in the protein level in Zucker fa/fa rats. Finally, we also analyzed the monomer/dimer equilibrium of nNOS and observed the presence of nNOS dimers in basal conditions in fa/fa rats, but not in the fa/+ rats.
**Conclusion:** Abnormalities in both the levels of nNOS expression and dimer/monomer equilibrium could explain the differential response of the β-cell to the two pharmacological inhibitors and the hypersecretion of insulin observed in the Zucker fa/fa rats.

## 566

**Galnon, a non-peptide galanin-receptor agonist, is a potent stimulator of insulin release in islets from healthy rats and diabetic GK rats**
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**Background and aims:** Galanin is a neurotransmitter peptide that suppresses insulin secretion. Recently, a non-peptide galanin receptor agonist, galnon, was discovered by application of a combinatorial library approach to galanin pharmacophores. The aim of the present study was to investigate how galnon affects insulin secretion from isolated pancreatic islets of healthy Wistar and diabetic Goto-Kakizaki (GK) rats.
**Materials and methods:** Isolated islets from Wistar and GK rats were batch incubated or perifused for studies of insulin release. Incubations were also performed to study the interaction on insulin release of galnon with galanin, with the galanin receptor antagonist M35, and with inhibitors of PKA and PKC. In addition, galnon effects were studied in islets depolarised by high concentration of KCl and diazoxide to keep the $K_{ATP}$ channels open, on islet intracellular $Ca^{2+}$ levels, $[Ca^{2+}]_i$, using Fura-2, as well as in the presence of the calcium channel blocker nimodipine.
**Results:** Galnon had potent stimulatory effects on insulin release in isolated Wistar rat islets; 100 μM of the compound increased the release 8.5 times ($p<0.001$) at 3.3 mM and 3.7 times ($p<0.001$) at 16.7 mM glucose. Also in islet perifusions, galnon augmented several-fold both acute and late phase of insulin response to glucose. In addition, galnon stimulated insulin release in islets from diabetic GK rats. These effects were not inhibited by the presence of galanin or the galanin receptor antagonist M35. Pretreatment of islets with pertussis toxin (PTX) did not suppress galnon-induced insulin release, suggesting that the galnon effect was not mediated by PTX-sensitive $G_e$ or $G_i$ proteins. The stimulatory effect of galnon at 16.7 mM glucose was inhibited by the PKA inhibitor H-89 (10 μM) by 37% ($p<0.05$) and the PKC inhibitor calphostin C (1.5 μM) by 95% ($p<0.001$). In islets of both control Wistar and GK rats, insulin release was stimulated by depolarisation of 30 mM KCl in the presence of 0.25 mM diazoxide, and 100 μM galnon further enhanced insulin release 1.5–2 times ($p<0.05$). $[Ca^{2+}]_i$ was increased in parallel with insulin release, and nimodipine suppressed insulin response to glucose as well as to galnon.
**Conclusion:** The non-peptide galanin agonist galnon is a potent stimulator of insulin release from islets of healthy Wistar and diabetic GK rats. The mechanism of this stimulatory effect does not seem to engage galanin receptors. Galnon-induced insulin release appears to involve modulation through the PKA and PKC systems and opening of L-type $Ca^{2+}$-channels, but the main effect of galnon is likely to be exerted at a step distal to these channels, i.e. at the exocytotic machinery of the B-cells.

## 567

**Effect of continuously available exenatide on β-cell secretion and insulin sensitivity in diabetic fatty Zucker rats**
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**Background and aims:** Continuously available exenatide (synthetic exendin-4) released from a depot formulation has previously been shown to dose-dependently improve fasting glucose and $Hb_{A1c}$ measured 28 days after injection into male Diabetic Fatty Zucker (ZDF) rats ($ED_{50}$'s of 106

and 155 µg/rat, respectively). The present study assessed the contributions of changes in β-cell function *versus* insulin sensitivity towards this effect.
**Materials and methods:** Single subcutaneous doses of a poly-lactide-glycolide microsphere suspension (3% peptide), a long-acting release formulation (LAR) containing 0, 1, 10 and 100 µg exenatide were administered into 9-week old animals assigned to groups (n=6,5,6,6, respectively) matched by mean $Hb_{A1c}$ prior to exenatide injection. After 28 days, fasted animals were anesthetized, infused with primed/continuous insulin (100 mU/kg/min), and plasma glucose clamped at 104 ± 4 mg/dL by glucose infusions varied in response to serial plasma glucose/lactate measurements. Insulin sensitivity index (M/I) was assessed as the glucose infusion rate required to maintain euglycemia *per* the ambient insulin concentration. At 180 min, insulin infusions were interrupted but glucose infusions maintained, and a glucose challenge was administered as an additional 1 g/kg intravenous glucose bolus. Glucose-driven β-cell secretion was assessed by changes in plasma C-peptide measured 5, 10, 30, 60, 90 min after the bolus.
**Results:** M/I, 45 minutes into the clamp, increased by 89.7 ± 38.0%, 70.6 ± 37.9% and 51.7 ± 9.0% *vs* vehicle controls at 1, 10 and 100 µg doses, respectively ($P<0.04$ at 100 µg dose). During the post-clamp glucose challenge, glycemic excursions were similar in all treatment groups (range 351–390 mg/dL) and the 90-min mean glucose values were similar (247 ± 8, 234 ± 8, 242 ± 4, 243 ± 4 mg/dL). C-peptide responses, measured as 90-min incremental AUC (nM·min) were 22.2 ± 5.5, 10.5 ± 4.5, 40.0 ± 15.5, 53.9 ± 12.6 for vehicle and 1, 10, and 100 µg doses respectively ($P<0.05$ *vs* vehicle for highest dose). Plasma lactate response to the glucose challenge, which can reflect aggregate insulin action, was dose-dependently elevated by prior exenatide LAR treatment (0.1 ± 12.8%, 11.7 ± 2.6%, 35.8 ± 8.0% *vs* control at 1, 10, 100 µg doses; $P<0.02$ ANOVA; $P<0.05$ for highest dose).
**Conclusion:** Chronic exposure (28d) to exenatide via a depot formulation (LAR) in ZDF rats is associated in the current study with improved β-cell secretion (assessed by C-peptide response), and increased insulin sensitivity (assessed by clamp). The proposal that increased overall insulin effect results from this combination of actions, and contributes to such dose-dependent glycemic reduction is supported in the present study by dose-dependent increases in post-challenge plasma lactate response.

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**Exenatide does not cause pancreatic islet cell proliferative lesions in rats and mice following 2-year exposure**
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**Background and aims:** Exenatide (synthetic exendin-4) and GLP-1 have been reported to increase β-cell mass in laboratory animals via stimulation of β-cell neogenesis and proliferation. The concern is that uncontrolled β-cell proliferation could occur with chronic exposure to any agent that stimulates GLP-1 receptors resulting in neoplastic lesions.
**Materials and methods:** In these 2-year carcinogenicity studies (following ICH guidelines) of exenatide in rats and mice (65 male and 65 female/group; Sprague-Dawley rats and CD-1 mice), animals received daily subcutaneous injections of placebo (vehicle, 2 groups) or exenatide (18, 70 and 250 µg/kg/day). Exenatide doses were equivalent to approximately 6, 25, and 90 times the systemic exposure expected from a 20 µg/day human dose. Pancreatic tissue samples from all animals were evaluated microscopically by board certified veterinary pathologists and subsequently peer reviewed. Statistical comparisons (Cochran-Armitage Trend Test, Fischer Exact Test, Peto Test) were made to each placebo group and to the combined placebo groups.
**Results:** There were no statistically significant differences or trends in occurrence of islet cell lesions between the placebo- and exenatide-treated groups.
**Conclusion:** In summary, daily exposure to exenatide over a 2-year period in the rat and mouse did not result in pancreatic islet cell tumors or other proliferative lesions.

| | Placebo | | Exenatide | | |
|---|---|---|---|---|---|
| **Dose (µg/kg/day)** | **0** | **0** | **18** | **70** | **250** |
| **MOUSE** | M (F) | M (F) | M (F) | M (F) | M (F) |
| Hyperplasia, islet cell | 1 (0) | 1 (0) | 0 (0) | 0 (1) | 0 (0) |
| Adenoma, islet cell, benign, primary | 0 (0) | 0 (0) | 0 (1) | 0 (0) | 0 (0) |
| Carcinoma, islet cell, malignant, primary | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| **RAT** | M (F) | M (F) | M (F) | M (F) | M (F) |
| Hyperplasia, islet cell | 13 (12) | 10 (1) | 13 (5) | 8 (3) | 8 (5) |
| Adenoma, islet cell, benign, primary | 2 (2) | 3 (1) | 3 (1) | 4 (2) | 5 (2) |
| Carcinoma, islet cell, malignant, primary | 0 (3) | 1 (1) | 0 (0) | 0 (0) | 0 (0) |

All data represent numbers of affected animals.

## 569

**Clock genes defect disrupts energy consumption and glucose metabolism; a study in cryptochrome-deficient (*mCry1*$^{-/-}$, *mCry2*$^{-/-}$) mice**
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A circadian rhythm of insulin secretion and action is required in the maintenance of plasma glucose concentrations within physiological boundaries. In mammals, a master circadian "clock" is located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus which can be entrained by environmental cues, in particular light-dark cycles, and it controls oscillator in peripheral organs through neural and hormonal signals. Keeping irregular hours as shift work and living during night time might have an impact on metabolic variables, and it may be also factor for metabolic disorders including diabetes. Recent discovery of clock gene has major molecular components in mechanisms responsible for circadian time keeping in mammals, but the cue controlling circadian rhythm of the autonomic nervous system and the metabolism is still unclear. We investigated energy consumption and glucose metabolism including glucose tolerance and insulin secretion by using clock genes deficient (*mCry1*$^{-/-}$*mCry2*$^{-/-}$) mice. In *mCry1*$^{-/-}$*mCry2*$^{-/-}$ mice kept in a 12:12 light-dark cycle, circadian activities including feeding were normal rhythm, ambient light being the most important cue for the SCN. However, circadian rhythmicity in oxygen consumption disappeared in these mice, and they were more hypermetabolic all the time and had lower adiposity than wild type mice. *mCry1*$^{-/-}$*mCry2*$^{-/-}$ mice exhibit impaired glucose tolerance due to decreased insulin secretion, and the insulin secretory responses to glucose in perfused pancreas were significantly impaired. These results revealed that the sympathetic nerve activity is elevated during all day to reduce adiposity and impair insulin secretion in *mCry1*$^{-/-}$*mCry2*$^{-/-}$mice. The defect of biological clock may disregulate the autonomic nervous system, leading to metabolic disorders including impaired insulin secretion.

# PS 40

# Insulin secretion in patients with Type 2 diabetes

## 570

**Assessement of 1st and 2nd phase of insulin secretion during OGTT and IVGTT**

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**Background and aims:** We recently assessed 1st and 2nd phase β-cell insulin secretion by applying the same model of glucose-induced insulin secretion to plasma glucose and C-peptide curves during both IVGTTs and hyperglicemic clamps. In the present study we have extended the same modeling strategy to standard OGTT (time 0'–120').
**Materials and methods:** We performed in 31 subjects (18 with normal glucose regulation [NGR], 7 with impaired glucose regulation [IGR], and 6 with newly diagnosed type 2 diabetes [T2DM]) a standard OGTT (blood samples for plasma glucose/C-peptide were collected every 5'–20' from 0' to 120'), and an IVGTT (12 g per $m^2$ of BSA; blood samples collected every 1'–20' from 0' to 180'–240') on 2 separate day. We have applied the same modeling strategy to both tests and obtained a fairly good fit of the data in both the IVGTT and the OGTT.
**Results:** We thus estimated first ($\sigma 1_{st}$) and second ($\sigma 2_{nd}$) phase insulin secretion during both tests. Results are normalized per $m^2$ of BSA. In the pooled data, OGTT $\sigma 1_{st}$ and $\sigma 2_{nd}$ (2996 ± 299 e 96.1 ± 7.37, respectively) were significantly higher ($p<0.01$) than IVGTT $\sigma 1_{st}$ and $\sigma 2_{nd}$ (467 ± 67 e 43.8 ± 4.3), reflecting the well known potentiating effect of oral glucose on β-cell response. Moreover, OGTT $\sigma 1_{st}$ and $\sigma 2_{nd}$ were positively and significantly correlated to IVGTT $\sigma 1_{st}$ and $\sigma 2_{nd}$ ($r=0.50$ e $r=0.52$, respectively; $p<0.01$ for both). Finally, in NGR, IGR and T2DM subjects OGTT $\sigma 1_{st}$ (3609 ± 430, 2439 ± 437 e 1807 ± 220) and $\sigma 2_{nd}$ (112 ± 9.5, 80.8 ± 13 e 66.2 ± 11.5, respectively) showed a similar declining pattern as the one observed with the IVGTT (624 ± 83, 427 ± 112 and 42.8 ± 27.8 for IVGTT $\sigma 1_{st}$; 44.8 ± 6.5, 48.3 ± 9.1 and 35.5 ± 5 for IVGTT $\sigma 2_{nd}$, respectively).
**Conclusion:** These data demonstrate that is feasible to assess 1st and the 2nd insulin secretion phase during a standard OGTT and provide a physiological tool to measure β-cell function in states of normal and/or alterated glucose regulation.

## 571

**Impaired first phase insulin response as predictor for postprandial glucose increment in newly diagnosed Type 2 patients in near normal metabolic control**

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**Background and aims:** Newly diagnosed patients with Type 2 diabetes are characterized by an impaired first phase insulin response. The impact of this defect as regards to the elevated postprandial blood glucose levels ($AUC_{Glucose\ 0-120\ min}$) is partly unknown.
The aim of the present study was to evaluate the correlation between postprandial blood glucose control and fractional β-cell response in diet treated Type 2 patients with near normal metabolic control.
**Materials and methods:** Twenty patients with Type 2 diabetes, mean age 62,7 ± 8,3 (SD) years, diabetes duration 1,1 ± 1,5 years, BMI 29,8 ± 4,8 kg/$m^2$ and HbA1 c 6,1 ± 0,8% were studied with an intraveneous glucose tolerance test (IVGTT) and a meal tolerance test (MTT). None of the patients had any late diabetic complications.
First phase insulin response ($AIR_G$) was assessed as $AUC_{insulin\ 0-10\ min}$ at the IVGTT and postprandial glucose increment was measured as $AUC_{Glucose\ 0-120\ min}$ at the MTT.
**Results:** The postprandial glucose excursions 2 hours after the meal was significantly correlated to first phase insulin response $AIR_G$ ($r = -0,45$, $p< 0,05$). Furthermore, the metabolic control defined by HbA1 c was negatively correlated to the first phase insulin response $AIR_G$ ($r = -0,42$, $p= 0,06$).
A stepwise multiple regression analysis, with measures of β-cell responsiveness at MTT and IVGTT revealed, that 43% of the variation in postprandial glucose increment is explained by variation in $AIR_G$ and peak insulin concentration during the first phase insulin response ($p< 0,01$).
**Conclusion:** The degree of impairment of first phase insulin response is a significant predictor of postprandial glucose increment in patients with early Type 2 diabetes with near normal metabolic control.

## 572

**Beta cell function and insulin resistance in relation to candidate genes of diabetes mellitus Type 2 in Czech population**

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**Background and aims:** Primary pathogenic causes of diabetes mellitus type 2 (DM2) are still not known, but the final common pathway is the decreased ability of peripheral tissues to respond to insulin (insulin resistance) and at the same time the loss of pancreatic beta-cell ability to compensate it by increase of insulin secretion. Relation between beta-cell function (BF) and insulin resistance (IR), resp. sensitivity (IS), describe disposition indices (DI=BF*IS).
**Materials and methods:** A total of 292 unrelated subjects with varying degrees of glucose tolerance were clustered on the basis of similarity of DI. DI was calculated from stimulated OGTT values of glycemia and insulin (BF: insulinogenic index and IS: Matsuda index, as we described previously in Vrbikova at al. Diabetes Care 25(7), 2002). The clusters consisting of subjects under 25 percentile (cluster 1) and above 75 percentile (cluster 2) of DI value distribution were compared according to biochemical parameters and anthropometric characteristics. The SNPs of INS VNTR (HphI), FABP2 (HpaI), PPAR gama (HgaI), Beta2AR (MvaI), Beta3AR (MvaI), Kir6.2 (BanII) and UCP1 (BclI) were determined by PCR-RFLP and genotype and allele frequencies were compared using nonparametric Mann-Whitney test. Statistical analyses were done by NCSS 2001 program.
**Results:** Cluster 1 consists of 58 subjects with sex ratio 23 M/35 F, family history of DM2 ratio (first degree) 29 yes/29 no and mean age 37.07 ± 12.55. Cluster 2 consists of 58 subjects with sex ratio 23 M/35 F, family history of DM2 ratio 17 yes/41 no and mean age 32.43 ± 10.87. The differences between clusters were not observed in age and sex ratio, but in cluster 1 there were significantly more subjects with positive family history of DM2 than in cluster 2 ($p=0.019$). Cluster 1 comprises subjects with significantly higher triacylglycerols (1.24 ± 0.63 vs. 0.84 ± 0.41, $p=0.0002$) and serum uric acid (292.17 ± 86.47 vs. 262.48 ± 74.69, $p=0.047$) than subjects in cluster 2. Men and women in cluster 1 had significantly higher WHR than in cluster 2 (men 0.89 ± 0.08 vs. 0.81 ± 0.04 and women 0.77 ± 0.09 vs. 0.72 ± 0.05; $p=0.0014$, resp. $p=0.015$). Women in cluster 1 had also lower muscle to subcutaneous fat ratio than women in cluster 2 (1.51 ± 0.55 vs. 1.89 ± 0.68, $p=0.028$). Differences in genotype and allele frequecies between clusters were observed only in SNP E23K of Kir6.2 gene (Fisher exact test). In cluster 1 compared to cluster 2 were identified genotype EE in 10 (20.8%) vs.18 (37.5%), genotype EK in 27 (56.3%) vs. 28 (58.3%) and genotype KK in 11 (22.9%) vs. 2 (4.2%) subjects, $p=0.014$. Allele frequencies in cluster 1 compared to cluster 2 were E 49% vs. E 66.7% and K 51% vs. 33.3%, $p=0.013$.
**Conclusion:** Relation between BF and IR, i e. DI, in non-diabetic subjects was calculated from the stimulated OGTT values. Clusters generated on the basis of DI value are sifnificantly different in some biochemical and anthropometric parameters. Higher frequency of K allele of Kir6.2 gene in cluster with lower DI is in agreement with described positive association of K allele with decreased BF. Higher frequency of the subjects with positive family history of DM2 in the cluster with lower DI revealed that stimulated DI is valid criterion for detecting of early stages of impaired glucose tolerance.
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## 573

**Reduced hepatic insulin extraction in normal weight and normal glucose tolerant first degree relatives of Type 2 diabetes mellitus patients as a further pathophysiological mechanism involved in the development of Type 2 diabetes mellitus**

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**Background and aims:** To study of early abnormality in the insulin handling by young, first-degree relatives of patients with T2DM (FDR).
**Materials and methods:** A hyperglycemic clamp (11.1 mmol/l for 115 min), followed by addition of GIP (2 pmol · kg · min, 60–115 min) and an arginine-bolus and -infusion (10 mg · kg · min, 90–115 min) was conducted on 14 healthy volunteers and 13 FDR´s, both groups with NGT. Hepatic insulin

extraction was calculated in three ways (a) from changes in ISR compared to changes in peripheral insulin concentrations, both expressed as percentages of their respective basal values, which was taken as 100%; (b) from a ratio of the incremental area under the ISR curve ($AUC_{ISR}$) to the incremental area under the peripheral insulin concentration curve ($AUC_{insulin}$) for each of the post-stimulatory periods of the clamp ($MCR_{insulin}$) and from the ratio of the $AUC_{ISR}$ to $AUC_{insulin}$ for the basal state; (c) from the molar ratio of the incremental area under the C-peptide curve ($AUC_{C\text{-}peptide}$) to the incremental area under the insulin concentration curve ($AUC_{insulin}$) for each of the poststimulatory periods of the clamp and from the ratio of the $AUC_{insulin}$ to $AUC_{C\text{-}peptide}$ for the basal state. Mann-Whitney rank-sum test for unpaired data was used for statistical comparisons.
**Results:** FDR´s were more insulin resistant ($HOMA_{IR}$) under basal conditions (p=0.003). FDR´s demonstrated significant global impairment in insulin secretion capacity which was not specific for one of the secretagogues. $MCR_{insulin}$ was significantly reduced in the group of FDR´s under basal conditions ($2.3 \pm 0.3$ vs. $4.0 \pm 0.3$ l × $min^{-1}$, p = 0.001) and in response to GIP-infusion ($1.4 \pm 0.2$ vs. $2.7 \pm 0.5$ l × $min^{-1}$; p = 0.029), but there was no general defect in $MCR_{insulin}$ in response to glucose and arginine. Fasting molar ratio of the $AUC_{C\text{-}peptide}$ to $AUC_{insulin}$ were higher in the control group compared to FDR´s ($13.2 \pm 1.8$ vs. $9.4 \pm 1.0$, p = 0.155). In response to GIP-infusion, the molar ratio decreased progressively in both group and were lower in the FDR´s compared to control subject ($2.9 \pm 0.5$ vs. $5.4 \pm 0.9$, p = 0.019), but there was no general defect in the molar ratio of the $AUC_{C\text{-}peptide}$ to $AUC_{insulin}$ in response to glucose and arginine (p = 0.793 and p = 0.193, respectively). The $HOMA_{IR}$ correlated negatively (p<0.01) with $MCR_{insulin}$ under basal conditions (r = –0.96) and under GIP infusion (r = –0.56).
**Conclusion:** Our study demonstrates the in vivo abnormality in the insulin handling in marginally insulin-resistant young normal-weight FDR´s. This group demonstrated relative and global impairment of insulin secretion in response to glucose, GIP and arginine, as a sign of limitation of β-cell secretion capacity. The reduction of hepatic insulin extraction under GIP-infusion in FDR´s was significantly greater than in control subjects and negatively correlated with the degree of insulin resistance. This suggests that decreased insulin clearance in response to GIP may represent a further pathophysiological mechanism involved in the development of type 2 diabetes mellitus.

## 574

**Hyperbolic relationship between beta-cell mass and fasting glycemia and accelerated islet loss in humans with Type 2 diabetes**
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Type 2 Diabetes (TTD) is characterized by a ~65% deficit in beta-cell mass, which appears to be due to increased beta-cell apoptosis. However, the relationship between beta-cell mass and the development of diabetes remains unclear in humans. Recent studies suggest that there is active islet turnover in adult humans, implying that increased beta-cell apoptosis in TTD might shorten islet survival time. We therefore posed the following **questions:** (1) Does the increased frequency of beta-cell apoptosis in TTD decrease islet survival time and (2) what is the relationship between beta-cell mass and fasting blood glucose concentrations in humans?
**Materials and methods:** Beta-cell replication and apoptosis (Ki-67 and TUNEL) and beta-cell fractional area were determined by morphometric analysis of tissue sections from autopsy pancreata from TTD (n=57) and non-diabetic (ND; n=48) cases. Fasting plasma glucose (FPG) was measured prior to the final illness. The mean islet survival time in TTD and ND was computed based on replication and apoptosis data.
**Results:** The computed survival time of islets was reduced from ~2.5 years to ~0.5 years (ND vs TTD) in body-mass index matched cases. There was a negative hyperbolic relationship between FPG and beta-cell mass in humans (r=0.5, p<0.0001), with a sharp point of inflection once beta-cell deficiency exceeds ~50% (cases on no treatment only included).
**Conclusion:** Increased beta-cell apoptosis in TTD causes a marked decrease in islet survival time that in turn most likely leads to the reported deficit in beta-cell mass. In humans a deficit in beta-cell mass of up to 50% appears to be tolerated but each further decrement in beta-cell mass induces a marked incerease of fasting plasma glucose concentrations. Efforts to overcome increased beta-cell apoptosis in TTD might lead to improved islet survival and thereby prevent the loss of beta-cell mass.

# PS 41
# IGT and obesity in children and twins

## 575

**Why girls are born lighter than boys, and the link to diabetes : the *'Gender Insulin Hypothesis'***
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**Background and aims:** Girls are born lighter than boys. The consistency of this observation across populations is striking, suggesting that it may have fundamental significance. Previous hypotheses relating low birth weight to subsequent diabetes have addressed *within*-gender differences in insulin resistance, but not *between*-gender. We propose that gender-specific genes affecting insulin sensitivity are responsible for the gender difference in birth weight – the more insulin resistant female fetus is less responsive to the trophic effects of insulin and is therefore smaller – and that this gender difference is retained throughout life.
**Materials and methods:** We drew on the EarlyBird cohort of 307 healthy school entrants (170 boys, 137 girls, mean age 4.9y, Plymouth UK) and their parents, and from recent literature, to examine between-gender relationships in birth weight, body mass, insulin resistance and metabolic disturbance. Fasting blood samples were taken for HOMA-IR and its metabolic correlates.
**Results:** The girls at 5y were 33% more insulin resistant than the boys, even after adjustment for differences in anthropometry, physical activity, body composition and energy expenditure. Furthermore, the girls had higher fasting triglycerides and lower HDL cholesterol and SHBG than the boys. Girls were of similar girth to the boys at 5y, but the fathers had acquired greater girth than the mothers (92.2 cm vs 80.8 cm) and were 7% more insulin resistant. Once adjusted for waist circumference, however, mothers were again more insulin resistant, by some 25%, suggesting that females are intrinsically more insulin resistant than males throughout life. Finally, girls are universally lighter than boys at birth, and cord blood insulin levels and insulin split fragments that mark for insulin resistance are higher in newborn girls than boys.
**Conclusion:** The *Fetal Insulin Hypothesis* predicts that the offspring of insulin resistant fathers would be lighter at birth, but has not been substantiated. The *Gender Insulin Hypothesis,* proposed here, examines gender differences and finds evidence for intrinsic (genetic) insulin resistance among females that can account for 1) the global predominance of females among young people with T2D 2) the higher prevalence of diabetes among adult males, but higher prevalence among females for any given BMI and 3) the most fundamental difference of all – birth weight. Insulin resistance may confer metabolic advantage in motherhood, ensuring nutrient supply to the fetus, and physical advantage on her sons by virtue of their heavier birth weight. The *Gender Insulin Hypothesis* also resolves some of the difficulties in evaluating candidate genes for insulin resistance. First, gender-specific genes have a clear impact on fetal growth. Second, one of the confounding effects on birth weight – gender – is entirely removed. Third, the prevalent metabolic disorders of young populations – insulin resistance and T2D – provide important phenotypic linkage. The search for genes underlying T2D might usefully focus on those accounting for the universal gender difference in birth weight.
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## 576

**Glucose intolerance as independent risk factor for cardiovascular disease in obese children**
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**Background and aims:** Observational data have established both hyperglycemia and obesity as risk factors for cardiovascular disease (CVD). According to prospective studies, there is a strong association between cardiovascular autonomic neuropathy (CAN) and major cardiovascular events. Abnormal blood pressure regulation is a characteristic feature of CAN. The aim of the present investigation was to compare risk of CVD between obese patients with and without impaired glucose tolerance (IGT)
**Materials and methods:** Three cohorts of patients were identified: Group 1: Seven obese children with IGT. Age (mean±SD): $13 \pm 3.7$ years, BMI: 30.5. Group 2: Eight obese children with normal glucose tolerance. Age: $14 \pm 2.2$ years, BMI: 33.8 kg/$m^2$. Group 3: Seven healthy children. Age: $12 \pm 3.1$ years, BMI:17.8 kg/$m^2$. Cardiovascular nervous function was evaluated by pos-

tural blood pressure testing with impedance cardiograph, a computerized noninvasive hemodynamic monitoring system. The following parameters were measured during rest, as well as 15 and 30 minutes after postural change: blood pressure, heart rate, cardiac output, stroke volume and peripheral vascular resistance.
**Results:** Resting systolic blood pressure (SBP) was highest in obese children without IGT. In response to postural change, drop in SBP was more pronounced in obese children, both with and without IGT. Resting heart rate was similar in all groups. Increase in heart rate, the physiological reaction upon posturation, was significantly less in obese children, especially in those with IGT ($p<0.05$). In response to postural change, stroke volume (SV) and cardiac output (CO) decreased in healthy children, while increased in both obese groups. Increase in SV and OU were more pronounced in obese children with IGT ($p<0.01$ and $p<0.05$ compared to controls). Peripheral vascular resistance increased in healthy children, while decreased in obese children upon posturation, more significantly in those with IGT ($p<0.05$).
**Conclusion:** According to orthostatic hypotension test cardiovascular autonomic nervous function is more affected in obese children with impaired glucose tolerance. Thus, glucose intolerance seems to be an additional risk factor for cardiovascular diseases in childhood obesity.
*Financial support was provided by Hungarian Research Foundations: OTKA TO43560 and ETT 346/2003*

## 577

**Reduced glucose tolerance in 7-year-old children with low birth weight and poor postnatal growth**

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**Background and aims:** Studies have demonstrated that low birth weight in combination with high postnatal growth can have a detrimental effect on insulin sensitivity during childhood. However, few studies have investigated the effect of low postnatal growth on glucose tolerance and insulin sensitivity in children born with low birth weights. Therefore, the aim of this study was to assess both these metabolic variables in 7-year-old children who were born with low birth weight and had experienced poor postnatal growth.
**Materials and methods:** A cohort of 468 children were chosen from the Birth to Ten study, an ongoing longitudinal survey of childhood health and welfare conducted in Johannesburg, South Africa. The children were chosen according to the criteria of African ethnicity and the availability of anthropometric data at birth, 1 and 5 years of age. A group of 240 children were randomly selected from this cohort and 152 (79 males, 73 females) subjects agreed to take part in the study. Fasting blood samples were taken and following an oral glucose load (1.75g per kg body weight) 30 and 120 minute blood samples were obtained. Insulin, proinsulin, des-31, 32 proinsulin, glucose and free fatty acid (FFA) levels were assayed in all blood samples. Weight and height were also measured. Insulin resistance was calculated using HOMA whilst beta cell function was assessed using the insulinogenic index (the ratio between the change in insulin and the change in glucose over the first 30 minutes of the OGTT). Children were split into tertiles of birth weight. Two groups were produced from the bottom tertile by subdividing them into those who had weights above the median for that tertile at age 7 (low-high group) and those with weights below the median (low-low group). The same process was applied to the children in the top tertile to produce high-high and high-low groups. This produced four groups of subjects each containing 23–25 individuals.
**Results:** Insulin, prohormone and FFA levels at fasting and during the OGTT did not differ between the four groups, and nor did fasting glucose levels. However, the area-under-the-curve for glucose levels during the OGTT was much higher in the low-low group ($708 \pm 28$ mM X minutes) than the other 3 groups (low-high, $604 \pm 21$; high-high, $627 \pm 19$; high-low, $594 \pm 22$; $p<0.05$ for each group versus low-low). The insulinogenic index was also lower in the low-low ($88 \pm 15$) compared to the other 3 groups (low-high, $297 \pm 133$; high-high, $163 \pm 39$; high-low, $140 \pm 12$; $p<0.05$ for each group versus low-low). HOMA values for insulin resistance were not different between the groups. However, if insulin resistance was assessed using the product of the areas-under-the-curve for insulin and glucose levels during the OGTT then differences were noted: low-low, $17.0 \pm 1.6$; low-high, $13.1 \pm 1.5$; high-high, $17.0 \pm 2.8$; high-low, $11.9 \pm 2.7$; $p<0.01$ for low-low and high-high versus high-low).
**Conclusion:** This data clearly shows that low birth weight in combination with poor postnatal growth in the first 7 years of life leads to raised postprandial glucose levels as a result of a reduced glucose-stimulated insulin response and some degree of postprandial insulin resistance. This data suggests that a combination of poor fetal and poor postnatal growth may increase the risk of glucose intolerance and Type 2 diabetes later in life.

## 578

**Impact of genetic versus non-genetic factors on plasma Insulin-like Growth Factor 1 (IGF-1) and IGF-Binding Protein 3 (IGFBP-3) levels in twins with normal and abnormal glucose tolerance**

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**Background and aims:** Studies indicate a role for IGF-1 in the control of glucose homeostasis including insulin action. A negative correlation between free IGF-1 and fasting glucose has been reported, and insulin sensitivity has been found positively correlated to free IGF-1 in the fasting state. Increased risk of developing impaired glucose tolerance (IGT) or type 2 diabetes mellitus (T2DM) has been shown to be associated with IGF-1 concentrations below the mean. Variations in circulating levels of IGF-1 and binding proteins are reported to be partly genetically determined. The aims of the present study were 1) To assess the hormone levels (IGF-1, IGFBP-3 and IGF-1/IGFBP-3 ratio) in twins with T2DM, IGT and normal glucose tolerance (NGT). 2) To study the impact of genetics versus non-genetic factors on the IGF-1 and IGFBP-3 levels (and IGF-1/IGFBP-3 ratio) in twins.
**Materials and methods:** A population-based cross-sectional study of 606 twins (303 twin pairs) identified through the Danish Twin Register. Of these, 125 pairs were monozygotic (MZ) and 178 were dizygotic (DZ). Plasma glucose and insulin concentrations were measured in the fasting state and during a standard 75-g oral glucose tolerance test. IGF-1 and IGFBP-3 were measured on fasting blood samples. BMI and waist/hip (W/H) ratio were obtained.
**Results:** Seventy-nine (79) individuals were diagnosed as having T2DM, 129 as IGT and 396 as NGT. The level of IGF-1 was higher in the T2DM group ($20.8 \pm 0.8$ nmol/l) than in the IGT and NGT groups ($18.2 \pm 0.5$ and $18.3 \pm 0.3$ nmol/l) ($p<0.05$). Both IGFBP-3 level and IGF-1/IGFBP-3 ratio were higher in the T2DM group ($3.5 \pm 0.1$ mg/l and $6.1 \pm 1.6$) compared to the NGT group ($3.3 \pm 0.0$ mg/l and $5.6 \pm 1.6$) ($p<0.05$). W/H ratio was $0.92 \pm 0.1$ in the T2DM group, $0.89 \pm 0.1$ in the IGT group and $0.86 \pm 0.1$ in the NGT group. BMI was $28.3 \pm 5.3$, $26.5 \pm 4.6$ and $25.3 \pm 3.8$ in the T2DM, IGT and NGT group respectively. W/H and BMI were significantly higher among the T2DM individuals than in both the IGT and NGT group ($p<0.05$). The group with diabetes were older ($67.7 \pm 4.39$ years) than the NGT group ($66.0 \pm 5.06$ years) ($p< 0.05$). Interclass correlations for MZ and DZ twins were used for estimation of heritability. Heritability (the proportion of variance attributable to genetic effects) was calculated for IGF-1 ($h^2=0.876$, $p<0.001$), IGFBP-3 ($h^2=0.712$, $p<0.001$) and IGF-1/IGFBP-3 ratio ($h^2=0.632$, $p<0.001$).
**Conclusion:** The results showed a higher level of IGF-1and IGFBP-3 and a higher IGF-1/IGFBP-3 ratio in T2DM twins compared with NGT twins, which to some extent may be explained by a higher BMI and W/H ratio in the T2DM patients. Nevertheless, the result may challenge the idea of an important role for low IGF-1 levels in the pathophysiology of T2DM. The results showed high heritability estimates for IGF-1 and IGFBP-3, supporting the notion of an important genetic control of IGF-1 and IGFBP-3 levels in plasma. Although the heritability estimate of the IGF-1/IGFBP-3 ratio was slightly lower than for total IGF-1, it indicated a major role for genetic factors in controlling perhaps also the free IGF-1 level in plasma.
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## 579

**Aetiology of regional fat distribution in twins – influence of genetic versus prenatal factors**

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**Background and aims:** Studies among rodents and humans have indicated an effect of an adverse prenatal environment and the later development of obesity. The aim was to estimate the relative impact of genetic versus prenatal factors (i.e. birth weight (BW) and zygosity) on regional fat distribution in twins.
**Materials and methods:** 108 MZ and 88 DZ twins in two age groups were DXA-scanned. To examine the associations between fat distribution, BW and zygosity a multiple regression analysis was developed allowing adjustment for zygosity status. Furthermore, intra-pair correlations and heritability estimates were calculated.
**Results:** Total-, trunk- and leg fat percent were all explained by age ($p<0.01$), sex ($p<0.0001$) and the interaction between BW and zygosity ($p<0.02$). The trunk-leg fat percent ratio was explained by age ($p=0.0025$) and sex ($p<0.0001$). In DZ twins, BW was negatively associated with total-, trunk- and leg fat percent. Surprisingly, in MZ twins, BW was positively associated with all fat parameters. The intra-pair correlations demonstrated significant non-genetic associations between BW and total fat percent ($r=0.27$, $p=0.033$) and leg fat percent ($r= 0.34$, $p=0.006$) among the young MZ twins. In old MZ twins, BW correlated significantly with leg fat percent ($r=0.40$, $p=0.009$). The heritability estimates were >1.0 for trunk fat percent, 0.83 for total fat percent, 0.39 for leg fat percent and 0.55 for trunk-leg fat percent ratio in the young twins and 0.73, 0.63, 0.42 and 0.38, respectively, in the old twins.
**Conclusion:** We demonstrate a zygosity-dependent influence of BW on fat distribution. The intra-pair correlations propose that this association is of non-genetic origin. Finally, the heritability estimates indicate a major genetic component in the aetiology of regional fat distribution.

## 580

**Overweight children: parents unaware and unconcerned (The EarlyBird Study)**
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**Background and aims:** Little is known about how accurately parents today are able to perceive the weight status of their children. No study to date has examined parental awareness of children's weight, in both mothers and fathers, nor assessed parental concern about their child's weight. The aims of this study were therefore to explore parents' awareness of overweight and obesity, both in themselves and in their seven year old children, and their degree of concern about weight, if any.
**Materials and methods:** Participants comprised 277 healthy children (mean age 7.4 years) and their parents (mean age 36 years), from the EarlyBird Diabetes Study. Body mass index (BMI) was measured in both parents and children. Overweight and obesity were defined as BMI >25 and >30 in parents, and in children as >91st and 98th centiles, respectively. Parents were asked, by written questionnaire, to estimate both their own and their child's weight on a five point rating scale ranging from „very underweight" to „very overweight". Further questions assessed the level of concern they felt, if any, about their child's weight. Possible answers ranged from „very worried about underweight" to „very worried about overweight".
**Results:** Children and parents were signifiantly heavier than UK norms. One third of mothers and half the fathers, who were either overweight or obese, rated themselves as „about right". Fifty-six percent of mothers and 66% of fathers perceived their overweight or obese child to be „about right". Even where the child was obese, 35% of mothers and 57% of fathers perceived him or her to be „about right". Parents were less likely to identify overweight and obesity in their sons than in their daughters: only 27% of overweight or obese boys were classified as at least „a little overweight", compared with 54% of the girls ($p=0.01$). Neither parents' own BMI nor their gender predicted their ability to assess their child's weight accurately. Sixty percent of parents of obese children expressed some degree of concern about their child's weight, but only 28% were concerned if the child was just overweight. Neither social class, nor level of parental education, nor family income, were related to BMI – actual or perceived.
**Conclusion:** Overweight and obesity are increasingly prevalent among all social classes, with overweight now widely accepted as the norm. As a result, the lay perception of the 'average' weight now conflicts with the clinician's definition of what constitutes a 'normal', that is, healthy weight. The implications are clear – recognition of excess weight is an essential first step in diabetes prevention.
*Supported by: Abbott, Astra-Zeneca, Child Growth Foundation, Diabetes Foundation, Diabetes UK, Ipsen, Unilever, EarlyBird Diabetes Trust*

## 581

**Longitudinal results from CHOPPS (Christchurch Obesity Prevention Project in Schools)**
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**Background and aims:** The CHOPPS project was a 12-month school based interventional study that focused at reducing the consumption of carbonated drinks. This study demonstrated a modest reduction in consumption of these drinks and prevented an increase in the prevalence of overweight and obesity. Our aim was to assess further anthropometric changes, 1 year after completion of the intervention.
**Materials and methods:** Anthropometric measurements including weight (Seca medical scale - 770), height (portable Leicester measure)
**Results:** 470 of 644 children (73%) of children with a mean age of 10.3 years ± 1 were measured. At 12 months the difference in prevalence of overweight and obesity between the groups was significant ($p= 0.048$, 95% CI: 2.2, 13.1). This is not repeated at 24 months ($p=0.17$, 95% CI -2.9, 10.5) although the prevalence of overweight and obesity remains consistent in the intervention group.
**Conclusions:** School based interventions are effective in the prevention of childhood obesity and the effect may last beyond the active intervention phase

Changes in prevalence of overweight and obesity

| | | Baseline | 12 Months | 24 Months |
|---|---|---|---|---|
| Control | Girls | 19% | 27% | 25% |
| | Boys | 21% | 27% | 25% |
| Intervention | Girls | 21% | 21% | 18% |
| | Boys | 20% | 20% | 20% |

## 582

**Childhood obesity in Cameroon: is Type 2 diabetes in adolescents around the corner?**
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**Background and aims:** Over the past few decades improvement in standards of living in developing countries has led to the adoption of "westernized" lifestyles favouring less physical activity and more high-fat diets. Exposure to fatty fast foods and sedentary leisure time activities put children and adolescents at risk of early obesity and hence early development of non-communicable diseases like type 2 diabetes.
This study was carried out to determine the prevalence of obesity and its determinants in school children in urban Cameroon. It also aimed to analyse the association between obesity, energy expenditure and glucose tolerance.
**Material and methods:** The study took place in two phases. The first phase was cross sectional and prospective on 4302 school children aged 5 to 16 years in Yaoundé. Data was collected on physical activity, nutritional habits anthropometrical and blood pressure measurements. The second phase was a nested case-control study on 23 obese children aged 10 to 16 years as cases and matched non-obese ones as controls. Fasting capillary blood glucose was measured and energy expenditure assessed by a Caltrac® accelerometer for both cases and controls. Body mass index above the 97$^{th}$ percentile for age and sex or above 30 kg/m$^2$ was used as a surrogate for obesity.
**Results:** The prevalence of obesity in our study population is 3%, with girls being more affected than boys regardless of age or school (3.1% versus 2.8%). The following risk factors for obesity were identified:
- high socio-economic level of the parents (OR=20.0 CI=3.2–829.1)
- small family size (OR=19.0 CI=1.0–8.3)
- diabetes in the father (OR=19.0 CI=3.0–789.5)
- hypertension in the father (OR=19.0 CI=3.0–789.5) and in the mother (OR=6.7 CI=2.0–35.0)
- obesity in the father (OR=5.7 CI=1.6–30.2) and in the mother (OR=7.0 CI=1.6–63.5)
- high weight of birth (OR = 10,5 CI = 2,6–92,4)

Total daily energy expenditure and daily physical activity energy expenditure evaluated by the accelerometer were higher in cases compared to con-

trols (2481 ± 35 calories versus 1899 ± 90 calories p = 0.001, 858 ± 106 calories versus 509 ± 59 calories p = 0.01 respectively). However, obesity was highly associated with physical inactivity. The mean fasting blood glucose of the cases was 4.8 ± 0.1 mmol/L against 4.5 ± 0.1 mmol/L for controls, p = 0.03. Obese children were more likely to have higher blood glucose values (>4.7 mmol/L) than controls (OR = 5 CI = 1.4–26.9), although there was no diabetic patient in both groups.
**Conclusions:** Physical inactivity increases substantially the risk of paediatric obesity in school children and could lead to type 2 diabetes in the adolescent.

## 583

**Prevalence of the metabolic syndrome in Hong Kong Chinese adolescents**
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**Background and aims:** We are seeing an increasing trend of type 2 diabetes being diagnosed at an earlier age among the adult population in Hong Kong. It is purported that these individuals already harbour the metabolic abnormalities including glucose intolerance during their adolescent years. However there is no local data on the prevalence of metabolic syndrome in this young age group. The purpose of this study is to estimate the prevalence of the metabolic syndrome using the National Cholesterol Education Program, Adult Treatment Panel III (NCEP) definition, among Hong Kong Chinese adolescents.
**Materials and methods:** 960 girls and 1156 boys of Chinese origin aged 11–20 years in secondary schools were examined. Data on anthropometric parameters and fasting blood samples were collected from these adolescents in the school setting. The NCEP definition for adult was used for classification of subjects with the metabolic syndrome except for obesity, which was defined by waist circumference ≥90$^{th}$ percentile.
**Results:** The overall crude prevalence of metabolic syndrome was 2.0% (2.5% in boys and 1.6% in girls). No significant difference in the prevalence of the metabolic syndrome between boys and girls was found. The prevalence was 10.9% for elevated triglyceride, 2.4% for low HDL-C, 11.3% for obesity, 0.3% for impaired fasting glucose and 20.2% for hypertension. Of these school children, 24.8% have one, 6.9%, 1.7% and 0.3% have two, three and four of the five abnormalities.
**Conclusion:** There is a high prevalence of hypertension among this adolescent population followed by obesity and dyslipidemia which are also common. These are worrying findings in our young and apparently healthy adolescents with strong public health implications, calling for more education in lifestyle modification.

# PS 42
# Metabolism – in vitro

## 584

**Effect of AMP, AICAR and metformin on activation status of human recombinant AMP kinase isoenzymes**
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**Background and aims:** Cellular effects of the antidiabetic agent metformin (Met) have been attributed recently, at least in part, to its activation of AMP kinase (AMPK), an enzyme with important roles in cellular glucose and lipid metabolism. The aim of this study was to examine if metformin has a direct effect on the activity of 4 human recombinant AMPK isoenzymes, including isoenzymes highly expressed in liver and muscle. Additionally, effects of the AMPK activator AICAR and Met on glucose output were compared in primary rat hepatocytes.
**Materials and methods:** GST-tagged human recombinant AMPK isoenzymes (α1β1γ1, α2β2γ1, α2β1γ2 and α2β2γ3) were generated using a baculovirus system and enzyme activity assessed via phosphorylation of a peptide derived from acetylCoA carboxylase-1. Cellular AMPK activation was measured by western blotting using phosphoepitope-antibodies for AMPK-Thr172 and ACC-Ser79. Basal and glucagon-stimulated hepatocyte glucose output was assessed in the absence or presence of AICAR (1 mM) or Met (2 mM).
**Results:** Compared to control, AMP exerted direct stimulatory effects on activity of AMPK isoenzymes α2β2γ1 (+370% with half-maximal effect at 0.8 μM) and α2β1γ2 (+210% with half-maximal effect at 1.3 μM), but α1β1γ1 and α2β2γ3 was unaffected (n=3). Similar to AMP, AICAR stimulates only the α2β2γ1 and α2β1γ2 isoenzymes (α2β2γ1 with half-maximal effect at 16 μM and α2β1γ2 with half-maximal effect at 12 μM). In contrast, Met (0.01–1 mM) had no effect on AMPK activity associated with any of the isoenzymes. Although both AICAR (1 mM) and Met (2 mM) can activate cellular AMPK (AMPK-Thr172 phosphorylation, ACC-Ser79-phosphorylation), basal hepatocyte glucose production was barely affected by Met, but was suppressed by 1 mM AICAR (basal 1.5±0.05; Met 1.2 ±0.03, p<0.05; AICAR 0.4± 0.02 mg/dl glucose/4 h, p<0.005). However, glucagon-stimulated glucose output (3.2±0.05 mg/dl glucose/4 h) was suppressed by both AICAR (0.21±0.01 mg/dl glucose/4 h, p<0.0005) and Met (1±0.02 mg/dl glucose/4 h, p<0.0005).
**Conclusion:** These data indicate lack of a direct effect of Met on several AMPK isoenzymes. The contrasting effects of AICAR and Met on basal hepatocyte glucose production may suggest differential actions of AMPK activators and metformin.

## 585

**A study about the function of aquaglyceroporins and their regulatory mechanisms in relation to metabolic syndrome**
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**Background and aims:** Aquaporin 7 (AQP7) and AQP9 are aquaglyceroporins expressed in adipocyte and hepatocyte, respectively. These AQPs have been shown to be upregulated during fasting and inhibited by insulin. AQP7 provides fat-derived glycerol into plasma and liver takes up glycerol via AQP9 for hepatic gluconeogenesis. In insulin-resistant animal, both AQP7 and AQP9 mRNA are increased, despite hyperinsulinemia. Paradoxically, thiazolidinedione, an insulin sensitizer, upregulates AQP7 in adipocytes. The aim of the study was to further clarify the regulations of AQP7 and AQP9 expressions in relation to metabolic syndrome.
**Materials and methods:** AQP9 expression was evaluated by immunoblot analysis after treatment of Hep G2 cells with various agents known to influence insulin sensitivity and adipocyte metabolism. We also administrated rosiglitazone (10 mg/100 g diet) to OLETF rats for 2 weeks to evaluate AQP regulation. RT-PCR analysis of AQP7 and immunoblot analyses of AQP2 and AQP9 were performed using adipose, renal, and hepatic tissues, respectively.
**Results:** Treatment of Hep G2 cells with oleic acid for 24 hr upregulated AQP9 protein in a dose-dependent manner ($p < 0.01$). Glycerol itself showed no regulatory role. Only high dose of rosiglitazone (100 uM) upregulated AQP9 protein ($p < 0.05$). AQP7 mRNA was increased in epididymal adipose tissue from the rosiglitazone-treated OLETF rats ($p < 0.05$) without

change of AQP9 expression in liver. In addition, there was a tendency to upregulation of AQP2 protein in outer medulla of kidney from the rosiglitazone-treated OLETF rats.
**Conclusion:** Our results suggest that free fatty acid regulate AQP9 expression directly and this may contribute to the pathophysiology of metabolic syndrome. Thiazolidinedione-induced upregulation of AQP7 in adipose tissue do not accompany the increase of hepatic AQP9, indicating thiazolidinedione may have salutary effects on AQP7 and AQP9 dysregulation in metabolic syndrome. Thiazolidinedione-related edema may be related with renal AQP upregulation.

## 586

**Activation of proteinkinase B by insulin is decreased in isolated hepatocytes from NZO-mice, a model system for the metabolic syndrome**
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**Background and aims:** In type-2-diabetes, increased glucose production by the liver due to insulin resistance is a central pathophysiological event and fasting hyperglycemia is the typical clinical correlate of this phenomenon. Insulin is known to inhibit hepatic glucose production by decreasing the expression of the two key regulatory gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and the glucose-6-phosphatase catalytic subunit (G6Pase). This effect of insulin is mainly mediated via activation of phosphatidylinositol (PI) 3-kinase and proteinkinase B (PKB). The pharmacological intervention in signaling events which regulate hepatic glucose production is generally regarded as a potential strategy for the treatment of the metabolic aberrations associated with this disease. However, the identification of the underlying signaling defects is a major prerequisite for a specific approach.
**Materials and methods:** In order to analyze insulin resistance of the liver and the underlying defect, we characterized the signal transduction of insulin in isolated hepatocytes from NZO/SJL-mice. The NZO-strain represents a polygenic mouse model with increased incidence for the development of obesity and type-2-diabetes with metabolic abberations closely resembling the metabolic syndrome in humans.
**Results:** In isolated hepatocytes from NZO-mice, activation of PKB by insulin was clearly decreased (EC50 5x10-8M vs. 5x10-9M insulin, max. induction 2,5-fold vs. 5-fold over basal) as compared to metabolically normal SJL control mice. Similarly, G6Pase enzymatic activity was increased in hepatocytes from NZO mice. In contrast, the levels of insulin dependent tyrosine-phosphorylation of the insulin receptor β-subunit were not significantly different between hepatocytes from both groups.
**Conclusions:** In summary, our data suggest that in this model (1) an endogenous defect resulting in insulin resistance of the liver is localized in the hepatocyte itself. Furthermore, these data suggest that (2) the defect in insulin signaling is localized on the level of PKB or upstream in the signaling cascade.

## 587

**The influence of gliclazide on the cellular glucose transport into lymphocytes of healthy subjects and of Type 2 diabetic persons – in vitro studies**
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**Background and aims:** Cellular glucose transport is involved in the pathogenesis of diabetic hyperglycemia and the reactivity to hypoglycemic drugs. The objectives of this experiment were to determine glucose transport before and after addition of gliclazide alone or with insulin to lymphocytes isolated from peripheral blood of healthy and Type 2 diabetic persons.
**Materials and methods:** The study group included 9 patients with newly diagnosed Type 2 diabetes treated with diet only and 9 matched healthy subjects with no family history of Type 2 diabetes, serving as a control group. Fasting blood samples were collected in heparinised tubes. Circulating lymphocytes were separated from whole blood by centrifugation with Gradisol L. Gliclazide alone or with insulin was added to 290 µL (3 × 105 cells) of cell suspension containing 3H-deoxy-D-glucose. At previously assigned time points 3H-deoxy-D-glucose uptake was stopped and the concentration of labelled glucose in the cells was measured by scintillation counting. Expression of Glut1 and Glut3 was investigated by an immunocytochemical method and Western-blot analysis.
**Results:** Mean maximal baseline values of 7546 ccpm, 16343 ccpm after adding gliclazide, and 16467 ccpm after adding gliclazide in insulin stimulated conditions were obtained in lymphocytes from the control subjects. In lymphocytes obtained from patients with Type 2 diabetes mean maximal values were 4689 ccpm baseline, 5680 ccpm after adding gliclazide, and 5578 ccpm after adding gliclazide in insulin stimulated conditions. The results revealed the significant differences between deoxy-D-glucose transport in lymphocytes obtained from patients with Type 2 diabetes and the control group ($P<0.05$). An immunocytochemical method and Western blot analysis showed the presence of Glut1 and Glut3 proteins in lymphocytes obtained from diabetics and healthy subjects.
**Conclusion:** Cellular glucose transport in lymphocytes in vitro is significantly impaired in Type 2 diabetes mellitus as shown in this study. It could be partially restored by adding gliclazide only – it was independent of insulin administration. These findings suggest a possible therapeutic role of gliclazide in correcting cellular glucose transport in Type 2 diabetes mellitus.

## 588

**RNAi-mediated glucose 6 phosphatase(G6Pase) and peroxisome proliferator-activated receptor gamma coactivator 1(PGC-1)depletion leading to the lowering of blood gluocse level in alloxan-diabetic mice**
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**Background and aims:** Synthetic short interfering RNAs (siRNAs) as well as vector-based siRNA expression systems have been used successfully to silent the gene expression in a variety of biological system. Many studies have shown that RNAi worked successfully on living mice. In this experiment, we evaluated the potential of RNAi as a therapy for diabetes by depressing gluconeogenetic enzyme.
**Materials and methods:** Synthetic short interfering RNAs (siRNAs) of Glucose 6-phosphatase and PGC-1 was constructed to siRNA-expressing plasmid DNA (pDNA). siRNA-expressing pDNA was injected to alloxan-diabetic mice by tail vein and blood gluose levels were monitored. G6Pase and PGC-1 mRNA were measured by RT-PCR.
**Results:** Post-prandial blood glucose levels were not changed either in control group or in PGC-1 and G6Pase siRNA pDNA injected alloxan-diabetic mice. However, when blood glucose levels were measured after six-hour-fasting, there were no change in the control group (225 mg/dL blood) but decrease of blood glucose levels were seen in group with PGC-1 (135 mg/dL blood) and G6Pase siRNA pDNA (126 mg/dL blood) injected alloxan-diabetic mice. Similary, when blood glucose levels were measured after twenty-hour-fasting, there were no change in the control group (195 mg/dL blood) but decrease of blood glucose levels were seen in group with PGC-1 (113 mg/dL blood) and G6Pase siRNA pDNA (116 mg/dL blood) injected alloxan-diabetic mice. G6Pase and PGC-1 mRNA levels in liver were not changed with injection of siRNA pDNA.
**Conclusion:** Gluconeogenesis may be inhibited successfully by silencing G6Pase and PGC-1 gene and siRNA pDNA in mice.

## 589

**Impact of cell glycogen content on modulation of hepatocyte glucose metabolism by pharmacological agents.**
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**Background and aims:** Pharmacological modulation of hepatic glucose metabolism represents a potential treatment in type 2 diabetic patients to prevent hyperglycaemia. Emerging new agents such as glucokinase activators (GKA), glycogen phosphorylase inhibitors (GPi) and GSK3 inhibitors (GSK3i) have been used to investigate the regulation of glycogen synthesis in cultured hepatocytes. Glucose up-take into liver is regulated by glucokinase (GK) while the net storage of glucose as glycogen is regulated by glycogen synthase (GS) and glycogen phosphorylase (GP). GS is inactivated by phosphorylation and GP is activated by phosphorylation. Protein phosphatase 1 (PP1) dephosphorylates both GS and GP thereby activating GS and inactivating GP. GSK3 phosphorylates and inactivates GS. GP exerts a tonic inhibitory effect on PP1 that is relieved by inhibition of GP. Consistent with this, in rat primary hepatocytes hepatic glycogen synthesis is stimulated by the GPi CP-91149. An additional level of regulation of glycogen

synthesis may be afforded by association of GS,GP and PP1 with the glycogen particle in complex with glycogen targeting subunit ($G_L$.) It is established in muscle that glycogen synthase activity is inversely correlated with glycogen content. We have investigated the effect of pharmacologically modulating GS and GP activities on glycogen synthesis in cultured primary hepatocytes with differing glycogen concentrations.
**Materials and methods:** Rat primary hepatocytes were cultured overnight in high or low glucose DMEM. GS activity in cell lysate was measured by the incorporation of UDP[1-$^3$H] glucose into glycogen. GP activity was measure spectrophotometrically. Glycogen was measured as glucosyl units after amylglucosidase digest. Glycogen synthesis was measured by the incorporation of [U-$^{14}$C] glucose into glycogen.
**Results:** In non-glycogen loaded hepatocytes GPi688 inhibited GP activity by 50% at 1 μM ($P<0.01$); 80% at 5 μM ($p<0.001$) and 90% at 10 μM ($P<0.001$).Conversely GPi increased GS activity 5 fold at 1 μM ($P<0.01$), 5 μM ($P<0.05$) and 10 μM ($P<0.001$). GSK3i activated GS in a dose dependent manner.However GKA1 did not significantly activate GS.A comprehensive analysis of GPi, GKA GSK3i and glycogen effects on glycogen synthesis will be presented.
**Conclusion:** We have confirmed that a GPi, acting at the dimer interface of GP, does indeed activate GS in cultured rat primary hepatocytes. Interestingly while GKA1 did not activate GS, as assessed in the cell lysate, this compound did stimulate glycogen synthesis.

# PS 43
# Metabolism – in vivo

## 590

**Assessment of postprandial glucose metabolism: conventional vs triple tracer method**
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**Background and aims:** The classic dual tracer method to measure postprandial glucose fluxes utilizes a glucose tracer ingested in the meal to label exogenous glucose while a second tracer is constantly infused to calculate both systemic appearance of ingested glucose ($Ra_{meal}$) and endogenous glucose production (EGP). The marked tracer nonsteady state that occurs with this method introduces errors and renders the estimates model dependent. To circumvent these problems, a triple tracer approach was proposed, which associates an oral tracer, [1-$^{13}$C] glucose, with two tracers, [6-$^3$H] glucose and [6,6-$^2H_2$] glucose, infused intravenously with a pattern that minimises the change in the plasma ratios of [6-$^3$H] glucose to [1-$^{13}$C] glucose and of [6,6-$^2H_2$] glucose to endogenous glucose, respectively. $Ra_{meal}$ and EGP measured with this approach are essentially model independent since nonsteady state errors are minimised. The purpose here was to compare $Ra_{meal}$ and EGP measured with the dual against the triple tracer method. The two methods have been simultaneously performed in normal humans, thus allowing to quantify the effect of nonsteady state error and model assumptions on postprandial fluxes.
**Material and methods:** 8 normals underwent a two day protocol, where the dual tracer method using a mixed meal labelled with [1-$^{13}$C] glucose and a continuous infusion of [6,6-$^2H_2$] glucose was associated with an intravenous infusion of [6-$^3$H] glucose, in patterns that in day-1 minimised the change in the plasma ratios of [6-$^3$H] glucose to [1-$^{13}$C] glucose and in day-2 that of [6-$^3$H]glucose to endogenous glucose. In day-1, $Ra_{meal}$ calculated by exploiting the continuous [6,6-$^2H_2$] tracer infusion (dual tracer) was compared with the reference estimated from the clamped [6-$^3$H] to [1-$^{13}$C] glucose ratio (triple tracer). Similarly, in day-2 EGP calculated by exploiting the continuous [6,6-$^2H_2$] tracer infusion (dual tracer) was compared with the reference estimated from the clamped [6-$^3$H] glucose to endogenous glucose (triple tracer).
**Results:** While the triple tracer approach provided essentially model independent estimates of $Ra_{meal}$ and EGP, results from the dual tracer approach depended on the adopted model. The two compartment model (2CM) performed better than a single compartment model (1CM) in estimating $Ra_{meal}$ and the initial splanchnic extraction (ISE) with 2CM (ISE= 10.9 ± 4.0%, Mean±SE) was closer than that with 1CM (ISE= 21.7 ± 3.7%) to the reference value (ISE= 3.7 ± 5.5%) albeit significantly higher in both cases. EGP profile with the dual method showed, at variance with the triple tracer method, a paradoxical pattern with an increase above basal in the early samples followed by a rapid decrease to negative values, with both 1CM and, albeit to a less extent, 2CM.
**Conclusion:** The traditional dual tracer method precludes a reliable simultaneous assessment of $Ra_{meal}$ and EGP if 1CM is used to analyse the data. The use of 2CM mitigates, but not completely avoids this problem.

## 591

**Glucose transport in human skeletal muscle using dynamic PET imaging of [11C]-3-O-methyl-D-glucose**
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**Background and aims:** Skeletal muscle utilizes the majority of insulin stimulated glucose metabolism. Insulin stimulation of glucose transport into skeletal muscle, activated through translocation of glucose transporters from cystosol to sarcolemma, is widely regarded as a pivotal process in control of rates of glucose metabolism and overall insulin sensitivity. The goal of the current study is to examine a new approach for specific and quantitative assessment of insulin action on glucose transport in human skeletal muscle. This approach is based upon using dynamic PET imaging of 3-O-methyl-D-glucose (3-OMG) labeled with $^{11}$C ([$^{11}$C]-3-OMG), a glucose analogue that can be transported but not further metabolized.
**Materials and methods:** 14, lean healthy volunteers with normal insulin sensitivity participated in these studies; 8 in basal conditions and 6 during

an euglycemic-iperinsulinemic clamp (30 mU/m$^2$ min$^{-1}$). [$^{11}$C]-3-OMG was given as a bolus injection and dynamic PET imaging of calf muscles was conducted for 90 min., with blood sampling to obtain arterial tracer activity. A physiological two-extra vascular compartment model with four rate constants ($K_1$, $k_2$ plasma-extracellular exchange; $k_3$, $k_4$ transport in and out of cell) was used in order to obtain quantitative information on glucose transport.
**Results:** Insulin, at circulating levels in the mid-physiological range, does not affect $K_1$ (0.018 ± 0.002 vs 0.018 ± 0.002 ml/ml/min) but significantly reduces by half the outward transport from the interstitial compartment ($k_2$: 0.137 ± 0.007 vs 0.074 ± 0.013 min$^{-1}$; p=0.02). In addition, a 6-fold significant increase were observed for the inward transport rate contrast to the tissue compartment ($k_3$: 0.012 ± 0.001 vs 0.073 ± 0.016 min$^{-1}$; p<0.001) while the outward transport from the tissue compartment increased during insulin-stimulated conditions by approximately 2-fold ($k_4$: 0.021 ± 0.003 vs 0.047 ± 0.009 min$^{-1}$). As a consequence of these changes in individual rate constants, the distribution volume of [$^{11}$C]-3-OMG in extracellular space, increased by 2.5-fold during insulin-stimulated compared to basal conditions (0.13 ± 0.01 vs 0.32 ± 0.06 ml/ml; p<0.01), and the intracellular distribution volume of [$^{11}$C]-3-OMG increased 5-fold (0.08 ± 0.01 vs 0.45 ± 0.06 ml/ml; p<0.001).
**Conclusion:** Additional dose-response studies in 7 lean adults revealed that supraphysiological insulin infusion (30 vs 120 mU/m$^2$ min$^{-1}$) further triplicate the inward transport rate contrast to the tissue compartment ($k_3$: 0.073 ± 0.016 vs 0.268 ± 0.07 min$^{-1}$; p<0.001). The high technical quality of the obtained images provides a solid basis for robust modeling. Our findings reveal a clear effect of insulin to modulate the rate constants pertaining to glucose transport in lean, healthy volunteers.
We conclude that dynamic PET imaging of uptake of [$^{11}$C]-3-OMG will provide a novel approach to in vivo imaging of the crucial insulin regulated step of glucose transport, a process crucial to the molecular physiology of insulin action in health and metabolic diseases.
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## 592

**The effect of insulin therapy on glucose production rate, glucose uptake, lipolysis and proteolysis in non-surgical ICU patients**
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**Background and aims:** Recent data suggest that the use of insulin to maintain intensive glycaemic control amongst surgical ICU patients can improve morbidity and mortality. The value of this procedure in non-surgical patients is not known. Current insulin therapy for non-surgical patients in many ICUs aims to keep plasma glucose below 9 mmol/l. The effect of this insulin therapy protocol on the catabolic response of critical illness, characterised by increased glucose production, increased lipolysis and proteolysis is unknown.
**Materials and methods:** A prospective study was conducted in seven critically ill non-surgical patients (6M:1F, age 64 ± 2.72 years; BMI 24.77 ± 0.77 kg/m$^2$) within 36 hours of their admission to the ICU. Patients with diabetes mellitus, pancreatitis, oral steroid use within 1 month of entering the ICU, or liver disease (LFTs > twice normal range), were excluded. All patients were receiving 20% dextrose intravenously to provide 25 kcal.kg$^{-1}$day$^{-1}$. Insulin was infused at a variable rate to maintain plasma glucose below 9 mmol/L. Glucose production rate (Ra) and rate of uptake (Rd), glycerol Ra (a measure of lipolysis) and leucine Ra (a measure of proteolysis) were measured with a 3 hour primed infusion of [6,6-2H2]glucose (170 mg, 1.7 mg.min$^{-1}$), [2H5]glycerol (0.15 mg/kg, 0.61 mg · kg$^{-1}$hr$^{-1}$) and [1-13C]leucine (1 mg/kg, 1 mg.kg$^{-1}$hr$^{-1}$). Steady state sampling was performed at 150 to 180 minutes. Results are compared with fasting values from an age and weight matched healthy control group. All data presented are mean ± SEM.
**Results:** The mean APACHE II score on the day of study was 15.43 ± 1.87. Mean plasma glucose at steady state was 7.95 ± 0.73 mmol · L$^{-1}$. The mean glucose infusion rate was 22.83 ± 0.74 μmol.kg$^{-1}$min$^{-1}$. The average insulin infusion rate was 4.31 ± 0.73 U.hr$^{-1}$ which achieved plasma insulin concentrations of 655.21 ± 181.38 pmol.L$^{-1}$. Endogenous glucose Ra was decreased (2.24 ± 3.02 μmol.kg$^{-1}$min$^{-1}$, p < 0.03) and glucose Rd was increased (25.08 ± 3.07 μmol.kg$^{-1}$min$^{-1}$, P < 0.0001) compared to fasting glucose Ra/Rd in the control subjects (8.7 ± 0.39 μmol.kg$^{-1}$min$^{-1}$). Glycerol Ra and concentration (1.66 ± 0.23 μmol.kg$^{-1}$min$^{-1}$ and 46.72 ± 12.25 μmol.L$^{-1}$ respectively) were reduced (p<0.02, P=0.07) compared to the control subjects (2.89 ± 0.47 μmol.kg$^{-1}$min$^{-1}$ and 70.03 ± 8.53 μmol.L$^{-1}$). Plasma NEFA concentrations were very low at 0.10 ± 0.03 mmol.L$^{-1}$ compared to fasting values in the control subjects (0.71 ± 0.09 mmol.L$^{-1}$, P<0.0001). Leucine Ra was 2.16 ± 0.31 μmol.kg$^{-1}$min$^{-1}$, which was not significantly different to fasting control subjects (1.71 ± 0.11 μmol.kg$^{-1}$min$^{-1}$, P=0.11).
**Conclusion:** In non-surgical ICU patients, the use of insulin to maintain glycaemic control below 9 mmol/l was effective in suppressing glucose production rate, increasing glucose uptake and decreasing lipolysis, but did not suppress proteolysis to levels below that seen in fasting control subjects. If full suppression of proteolysis is to be of benefit in this catabolic group of subjects, then higher doses of insulin will be necessary.
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**In situ lipolytic regulation in subjects born small for gestational age**
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**Background and aims:** Subjects born small for gestational age (SGA) who are prone to develop insulin resistance in adulthood display an abnormal development of the adipose tissue during fetal and post-natal life. Since the lipolytic activity of the adipose tissue is critical in the development of insulin-resistance, the purpose of this study was to investigate whether SGA itself might affect lipolysis regulation.
**Materials and methods:** We studied the effect of catecholamines, by local injection of isoproterenol, and the effect of insulin, using 2-step infusion at 8 and 40 mU/m$^2$/min, on the in situ lipolysis of the abdominal subcutaenous adipose tissue of 46 subjects born either SGA or appropriate for gestational age (AGA), using the microdialysis technique.
**Results:** Isoproterenol infusion increased dialysate glycerol concentration in the adipose tissue in both groups, but the effect was1.5 fold greater in the SGA than in the AGA group (120 ± 16 vs 81 ± 16% p=0.02). Interestingly, plasma FFA concentrations consequently increased by 20 ± 7% in the SGA group (p=0.04) whereas no significant increase was observed in the AGA group. The antilipolytic action of insulin on dialysate glycerol concentration was similar in the SGA and AGA groups during the 1st (−41 ± 6 vs −32 ± 10%, p=0.17) and the 2nd (−63 ± 18 vs −58 ± 28%, p=0.12) steps of insulin infusion.
**Conclusion:** In summary subjects born SGA demonstrated an increased response to isoproterenol whereas the antilipolytic effect of insulin was similar to what observed in the AGA group. Considering the deleterious effect of FFA on insulin-sensitivity, the hyper reactivity to catecholamines might be regarded as an additional deleterious component of the insulin-resistance associated with SGA. In contrast the antilipolytic action of insulin does not seem to play a major role in the long-term metabolic complications associated with reduced fetal growth.

Clinical characteristics of the study population

| | SGA (n=23) | AGA (n=23) | p value |
|---|---|---|---|
| Birth weight (g) | 2766 (130) | 3313 (230) | <0.0001 |
| Age (yr) | 23.3 (3.3) | 23.2 (3.4) | 0.95 |
| BMI (kg/m$^2$) | 21.7 (2.3) | 21.5 (1.7) | 0.47 |
| Waist/hip ratio | 0.81 (0.06) | 0.82 (0.08) | 0.93 |

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**Effects of catecholamines on skeletal muscle and adipose tissue lipolysis**
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**Background and aims:** Deposition of triglycerides (TG) in skeletal muscle, is considered to be of importance in the pathogenes of insulin resistance. The hormonal regulation of TG-hydrolysis in skeletal muscle is however, not fully elucidated. In this study, we investigated the effect of catecholamine stimulation on skeletal muscle (SM) lipolysis in vivo using microdialysis. Comparison was made with corresponding effects on adipose tissue (AT) lipolysis.
**Materials and methods:** Glycerol levels (index of lipolysis) were registered in healthy, normal weight subjects in m. gastrocnemius and in abdominal

subcutaneous AT during a hyperinsulinemic, hypoglycemic clamp (n=13), and in response to in situ perfusion of adrenaline and noradrenaline ($10^{-10}$M–$10^{-5}$M) (n=12), respectively. Local tissue blood flow was monitored with the ethanol perfusion technique.
**Results:** *Hypoglycaemia:* Basal fractional glycerol release (difference between tissue and arterial and glycerol) was 4 times higher in adipose tissue than in skeletal muscle. In response to iv insulin infusion (plasma insulin 708 ± 72 pmol/l), the fractional glycerol release in AT was reduced by half, whereas it remained unchanged in SM. In response to hypoglycaemia (2.6 ± 0.1 mmol/l for 30 min), plasma adrenaline and noradrenaline increased 10-fold and 2-fold, respectively. At the same time, the fractional glycerol release increased markedly in both AT (returned to basal levels) and SM (70% increase) ($p<0.0001$ in both tissues). No significant changes in AT and SM blood flow were registered. *In situ catecholamine perfusion:* In AT, tissue glycerol increased significantly ($p<0.0001$) above basal levels at $10^{-7}$M of both adrenaline and noradrenaline, and maximum stimulation was seen at $10^{-6}$–$10^{-5}$M of the two catecholamines. In SM a significant ($p<0.0001$) elevation of tissue glycerol was found at $10^{-7}$M of adrenaline and $10^{-6}$M of noradrenaline, respectively. In both AT and SM, the maximum stimulating effect on tissue glycerol was significantly ($p<0.01$) higher with adrenaline than with noradrenaline (4-fold in AT, 2-fold in SM).
**Conclusion:** Our findings indicate major differences in lipolysis regulation between SM and AT. First, no antilipolytic effect of insulin was evident in SM. Secondly, in SM as in AT, catecholamines stimulate lipolysis. In AT, the sensitivities to adrenaline and noradrenaline are comparable. By contrast, in SM, adrenaline is more potent than noradrenaline. Hence, adrenaline appears to be the prime endogenous catecholamine in the regulation of SM lipolysis in humans.

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### Hindered transcapillary transport of insulin to muscle Tissue after oral glucose 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 during glucose/insulin infusion. However, it is not clear whether the delay in insulin distribution could be reproduced during more physiological conditions.Therefore, the aim of the present study was to follow the distribution of insulin to skeletal muscle after an oral glucose load in obese subjects.
**Materials and methods:** 10 lean and 10 obese males (BMI 23 ± 0.6 vs. 33 ± 1.2 kg/m$^2$, $p<0.001$) were investigated during an oral glucose tolerance test (OGTT). Insulin measurements in muscle interstitial fluid (with the microdialysis technique) were combined with forearm A-V catheterization and blood flow measurements with vein occlusion pletysmography.
**Results:** During the first 60 min after the OGTT, interstitial insulin was significantly lower (35–55%) than plasma insulin in the obese group ($p<0.05$). In lean subjects no significant difference between interstitial and plasma insulin levels was found during the first hour after the oral glucose load. Muscle glucose uptake during OGTT was similar in the two groups, but obese subjects had a significantly higher p-insulin level at 90–120 min after oral glucose (398 ± 57 vs. 224 ± 37 pmol/L in controls, $p<0.05$). Also interstitial insulin was higher in the obese group 230 ± 35 vs. 146 ± 21 pmol/L in lean controls, $p<0.05$, at 90–120 minutes. Permeability surface area (PS) for glucose increased in the lean group from 0.2 ± 0.1 ml/min/100g, at 0 min, to 0.5 ± 0.1, at 60 min, $p<0.05$, but not in the obese group (PS 0.2 ± 0.1 at 0 min and 0.3 ± 0.1 at 60 min, ns).
**Conclusion:** There was a significant gradient between plasma insulin and muscle interstitial insulin during the first hour after OGTT in the obese group in contrast to that in lean subjects, suggesting a delayed transcapillary transport in obese subjects. The hindered transport of insulin over the capillary wall in obese subjects after an oral glucose load may be due to a defect in insulin- mediated capillary recruitment as suggested by the blunted increase in PS for glucose. It may also be suggested that the higher plasma insulin response in obese subjects during an OGTT may be compensation not only for insulin resistance in the muscle but also for a diminished insulin distribution to peripheral tissues.

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### Ghrelin-insulin interelationship during euglucaemic clamp in insulin resistant state

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**Background and aims:** The existence of temporal relationship between ghrelin and insulin was recently shown, leading to suggestion that postprandial hyperinsulinemia might inhibit ghrelin secretion during meal absorption. The aim of our study was to investigate the relationship between insulin and ghrelin during euglycemic hyperinsulinemic clamp in a state of insulin resistance: polycystic ovary syndrome (PCOS).
**Materials and methods:** In that order, euglycemic hyperinsulinemic clamp was performed in 16 patients: 8 lean (mean age: 21.87+/–2.74 yrs; BMI: 20.42+/–1.39 kg/m$^2$) and 8 obese (age: 26.35 +/–8.06; BMI: 33.04 +/–5.31 kg/m$^2$) patients with PCOS. In all patients plasma insulin (RIA INEP, Zemun, mU/l) and total plasma ghrelin (Phoenix, USA, pg/ml) were determined at 0, 30 45, 60, 90, 100, 110, 120 and 240. minute (120 minutes after stopping the insulin infusion).
**Results:** Following results were obtained in lean vs. obese PCOS patients: basal insulin (33.89 +/–16.87 vs. 27.11 +/– 5.61 mU/l, $p > 0.05$), M index (2.80 +/– 0.53 vs. 1.62 +/– 0.13 mg/kg/min, $p < 0.05$), insulin plateau (88.42 +/– 23.82 vs. 117.57 +/– 17.98 mU/l, $p > 0.05$) and ghrelin (0 minute: 57.01 +/–8.93 vs. 45.65 +/– 10.37 pg/ml, $p > 0.05$; 30. minute: 51.62 +/– 6.14 vs. 41.41 +/– 7.89, $p > 0.05$; 45. minute: 52.74 +/– 4.54 vs. 40.11 +/–6.72, $p > 0.05$; 60. minute: 46.19 +/– 2.61 vs. 39.61 +/– 8.31, $p > 0.05$; 90. minute: 47.89 +/–4.32 vs 37.39 +/–5.63, $p > 0.05$; 120. minute: 47.36 +/– 5.12 vs. 35.00 +/– 5.85, $p > 0.05$, 240. minute: 58.50 +/–9.79 vs. 59.55 vs. 14.30, $p > 0.05$). Plasma ghrelin decreased significantly during insulin infusion in both lean (57.01 +/–8.93 vs. 41.89 +/–3.04, $p < 0.05$) and obese (45.65 +/–10.37 vs. 33.54 +/–6.0 pg/ml, $p < 0.05$) PCOS patients. Ghrelin levels returned to near basal values 2 hours after stopping the insulin infusion in both group of patients: lean PCOS (58.5 +/–9.79 pg/ml) and obese PCOS (59.55 +/–14.30 pg/ml). There was no correlation between M values and basal ghrelin levels in both lean ($r = -0.43$, $p > 0.05$) and obese ($r = 0.051$, $p > 0.05$) PCOS patients.
**Conclusion:** In conclusion, insulin infusion in our study during clamp acutely suppresses ghrelin plasma levels independently of insulin resistance levels.

# PS 44
# Steroids and 11β-HSD

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**Splanchnic cortisol production occurs in humans: evidence for tissue specific 11-β hydroxy steroid dehydrogenase (11β HSD) Type 1 activity**

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**Background and aims:** Glucocorticoids are potent regulators of protein, fat and carbohydrate metabolism. The aim of the study was to determine if local cortisol production occurs within the splanchnic bed and leg in humans.
**Material and methods:** 11 non-diabetic subjects were studied using the hepatic/leg catheterization method and an infusion of cortisol (F) labeled with 4 deuteriums (4DF) as proposed by Andrews et al (JCEM, 2002).
**Results:** In the fasting state, there was net release ($p<0.05$) of cortisol from the splanchnic bed ($6.1 \pm 2.6$ μg/min) and net uptake ($p<0.05$) by the leg ($1.7 \pm 0.7$ μg/min). This, along with cortisol production by other tissues (e.g. the adrenals) resulted in total body cortisol appearance of $18.1 \pm 1.9$ μg/min. Fractional splanchnic 4DF extraction averaged $12.9 \pm 1.3\%$ ($p<0.001$), splanchnic cortisol uptake $14.8 \pm 2.0$ mg/min ($p<0.001$), and splanchnic cortisol production $22.2 \pm 3.3$ μg/min ($p<0.001$). On the other hand, fractional leg 4DF extraction averaged $5.6 \pm 1.8\%$ ($p<0.02$), leg cortisol uptake $2.3 \pm 0.7$ μg/min ($p<0.01$), and leg cortisol production $0.4 \pm 0.4$ μg/min, which did not differ from zero. Since 4DF loses a deuterium during conversion via 11-β HSD type 2 to 3D cortisone (E) which in turn generates 3DF when 3DE is converted to cortisol via 11-β HSD type 1, 3DF production can be used as an index of 11-β HSD type 1 activity. Net splanchnic 3DF release ($3.9 \pm 0.4$ μg/min) and splanchnic 3DF production ($7.1 \pm 0.7$ μg/min) occurred ($p<0.01$) in all subjects whereas leg 3DF production ($0.04 \pm 0.08$ μg/min) did not occur indicating substantial 11-β HSD type 1 activity in the splanchnic bed but not the leg. During a euglycemic hyperinsulinemic (~150 pmol/l) clamp, net splanchnic cortisol balance decreased to rates that no longer differed from zero ($0.8 \pm 3.0$ μg/min) due to a slight but non-significant increase in splanchnic cortisol uptake ($17.9 \pm 3.4$ μg/min) and decrease in splanchnic cortisol production ($17.2 \pm 3.3$ μg/min).
**Conclusions:** In summary, a) net splanchnic release of cortisol occurs in humans, b) conversion of 4DF to 3DF indicates substantial 11-β HSD type 1 activity within the splanchnic bed, c) local splanchnic cortisol production in non-diabetic humans can approximate that present in the remainder of the body. We conclude that 11-β HSD type 1 causes tissue specific synthesis of cortisol within the splanchnic bed presumably exposing the liver to glucocorticoid concentrations that are substantially higher than those present in the peripheral circulation.
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**Primarily increased expression of 11β -hydroxysteroid dehydrogenase Type 1 mediates an increased sensitivity to cortisone in myotubes established from Type 2 diabetic subjects**

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**Background and aims:** Alterations in glucocorticoid hormone metabolism in skeletal muscle have been suggested to contribute to the pathogenesis of the metabolic syndrome. This study aims to investigate how human myotubes established from healthy subjects and type 2 diabetic subjects express glucocorticoid receptor (GR) and 11β hyrdoxysteroid dehydrogenase (HSD1 and HSD2), and to investigate how chronic exposure to cortisone and cortisol affects basal and insulin-stimulated glucose transport.
**Materials and methods:** Human myotubes established from control subjects and T2D subjects were allowed to differentiate for eight days. The last four days, myotubes were exposed to various combination of cortisone, cortisol, insulin and the HSD inhibitor carbenozolone. mRNA of GR, HSD1, HSD2 and glucose uptake was determined.
**Results:** We found that myotubes established from T2D subjects show an increased expression of mRNA HSD1 compared to control myotubes ($0.016 \pm 0.003$ vs $0.008 \pm 0.002$ arb.units, $p<0.03$), but with no differences in mRNA GRα. mRNA HSD2 was not expressed. Cortisone reduced basal glucose uptake in diabetic myotubes to $0.70 \pm 0.04\%$ of glucose uptake without exposure and in control myotubes to $0.92 \pm 0.06\%$, more pronounced in diabetic myotubes ($p<0.05$), and insulin-mediated glucose uptake in diabetic myotubes $0.57 \pm 0.05\%$ and in control myotubes $0.77 \pm 0.17\%$, with a further increase in mRNA HSD1 suggesting that cortisone enhances its own conversion. The cortisone effect could be abolished by the HSD1 inhibitor carbenoxolone. Moreover, mRNA HSD1 expression was negatively correlated with glucose uptake ($r = -0.31$, $p<0.001$). In contrast, myotubes exposed to cortisol expressed an equal, reduced glucose uptake in diabetic and control myotubes under acute insulin stimulation ($0.69 \pm 0.04$ and $0.63 \pm 0.02\%$, $p>0.05$) and at basal ($0.71 \pm 0.04$ and $0.64 \pm 0.06\%$, $p>0.05$).
**Conclusion:** Our study shows that diabetic myotubes express an increased sensitivity to cortisone mediated by an increased mRNA HSD1 expression, mRNA HSD1 enhancement, and followed by a reduced basal and insulin-medicated glucose uptake. Thus, the diminished glucose uptake seen in T2D subjects in vivo may partly be secondary to a primarily increased sensitivity to cortisone in skeletal muscle based on an increased HSD1 expression. Increased HSD1 activity in skeletal muscles may play a crucial role in the pathogenesis of the metabolic syndrome and could be a new important target in the treatment of insulin resistance.

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**Relative functional hypercorticism as an etiologic factor of insulin resistance**

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**Background and aims:** Relative functional hypercorticism (RFH) is a result of altered sensitivity of the hypothalamic-pituitary-adrenal axis and is characterized with increased sensitivity to stimulation, as well as reduced cortisol suppression (CS) after the 90th min of OGTT (conclusion from author's doctors thesis), visceral body fat distribution (VBFD) and is associated with insulin resistance (IR). Healthy nonobese subjects have CS higher than 50% of the basal C levels after the 90th min of OGTT. We discovered the relationship of IR with RFH determined by reduced percentage of CS (CS%), as well as with increased adrenal glands magnitude, and VBFD.
**Materials and methods:** Healthy women (n=123), with mean age ($34,59 \pm 11,97$ yr.) and BMI ($35,25 \pm 10.23$ kg/m$^2$) were examined and were divided in 3 groups according to HOMA values (H): 1st gr. H<4, 2nd gr. H=(4–8), and 3rd gr. H>8. The following parameters were determined: insulin resistance (IR) by HOMA values, the CS% during OGTT, adrenal glands volume (AGV) by echo tomography, BMI, WHR and waist to thigh ratio (WTR). CS% indicates the percentage of the post suppressive cortisol (C) value reduction from the basal C level. Greater% of CS means greater reduction of basal C levels during the test and better C suppressibility.
**Results:** CS% was ($68,21 \pm 5,81\%$) in the 1st, ($54,38 \pm 3\%$) in the 2nd and ($38,53 \pm 6,43\%$) in the 3rd gr. and was significantly different between the groups ($p<0,023$). CS% correlated significantly ($p<0,017$) with H, also correlated significantly ($p<0,0001$) with the right AGV, and with left AGV ($p<0,009$), as well as with BMI, WHR and WTR ($p<0,0001$). Right AGV was ($2,19 \pm 1,32$ cm$^3$) in the 1st, ($3,56 \pm 1,99$ cm$^3$) in the 2nd and ($3,78 \pm 2,12$ cm$^3$) in the 3rd gr. Left AGV was ($3,02 \pm 1,15$ cm$^3$) in the 1st, ($3,88 \pm 1,34$ cm$^3$) in the 2nd and ($5.83 \pm 3.86$ cm$^3$) in the 3rd gr. AGV correlated significantly ($p<0,001$) with WHR and WTR. H correlated significantly ($p<0,0001$) with the volumes of both adrenals. WHR was ($0,85 \pm 0,05$) in the 1st, ($0,95 \pm 0,09$) in the 2nd and ($1.1 \pm 0,1$) in the 3rd gr. WTR was ($1,43 \pm 0,07$) in the 1st, ($1,61 \pm 0,07$) in the 2nd, and ($1,89 \pm 0,1$) in the 3rd gr. WHR and WTR values were significantly different ($p<0,0001$) between the groups and correlated significantly with HOMA values ($p<0,001$). BMI was ($28,65 \pm 8,38$ kg/m$^2$) in the 1st, ($38,48 \pm 8,29$ kg/m$^2$) in the 2nd, and ($42 \pm 8,67$ kg/m$^2$) in the 3rd group. BMI was significantly different between the groups ($p<0,0001$) and correlated positively ($p<0,024$) with H.
**Conclusion:** Reduced CS% after the 90th minute of OGTT was significantly positively related to increased IR, reduced insulin sensitivity. Increased IR determined by HOMA was also significantly positively related to increased adrenal glands volume, and evidently with visceral obesity. RFH is an etiologic factor for IR and the metabolic syndrome.

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**The effects of dehydroepiandrosterone (DHEA) replacement on insulin sensitivity, body composition, and skeletal muscle physiology in hypoadrenal women**

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**Background and aims:** Dehydroepiandrosterone (DHEA) and its sulphated ester (DHEAS) are the most abundant steroid hormones found in the circulation. The physiological function of these adrenally produced hormones has yet to be determined. DHEA replacement is not part of the standard of medical care for hypoadrenal subjects. Previous work has shown that DHEA can lead to changes in body composition, insulin sensitivity and skeletal muscle strength.
**Materials and methods:** In a single centre, randomised, double blind, placebo controlled, cross over study 33 hypoadrenal women (±SD) (mean age 47.9 ± 15.6 years) were given 50 mg of DHEA as a single daily dose and identically encapsulated placebo, for 3 months each. Subjects had assessments of body composition by DEXA, physical function by bicycle $VO_2$ max testing, and skeletal muscle strength testing. Insulin sensitivity was assessed by hyperinsulinaemic euglycaemic clamping. Skeletal muscle needle biopsies were taken to assess the effects of DHEA replacement on mitochondrial enzyme activity, and synthesis rates of mixed muscle protein, mitochondrial, and sarcoplasmic proteins.
**Results:** There were no differences in body composition as assessed by measurements of fat mass, fat free mass and bone density. Insulin sensitivity was significantly improved with DHEA compared with placebo. The M value was higher with DHEA supplementation (1.34 ± 0.53 vs 1.22 ± 0.52 mg /min/KgFFM, p=0.02) despite comparable glycaemic control. Fasting plasma insulin and glucagon levels were significantly lower after 12 weeks of DHEA supplementation (7.07 ± 4.35 vs 8.88 ± 5.81 μU/ml, p<0.01 and 51.06 ± 17.2 vs 56.03 ± 22.85 pmol/l, p=0.04 respectively). There was a strong trend to lowering fasting glucose (4.67 ± 0.54 vs 4.83 ± 0.58 mmol/l, p=0.06). This study also demonstrated significant reductions in total cholesterol, (4.62 ± 0.9 vs 5.23 ± 0.83 mmol/l, p<0.01), triglycerides, (1.52 ± 0.7 vs 1.70 ± 0.80 mmol/l, p=0.02), and HDL cholesterol, (0.97 ± 0.32 vs 1.09 ± 0.33 mmol/l, p<0.01) with DHEA vs placebo.The present study showed no change in mitochondrial enzyme activity (cytochrome c oxidase 89.22 ± 22.48 vs 96.35 ± 13.11 uU/g protein, p=0.55, and citrate synthase 137.07 ± 36.63 vs 142.11 ± 35.66 uU/g protein, p=0.46, DHEA vs placebo respectively). There were no changes in skeletal muscle protein synthesis rates with DHEA vs placebo, (mixed muscle protein 0.057 ± 0.02 vs 0.0529 ± 0.009 %/hr, p=0.38, mitochondrial protein 0.0569 ± 0.02 vs 0.053 ± 0.087%/hr, p=0.38, sarcoplasmic protein 0.0363 ± 0.009 vs 0.0378 ± 0.006%/hr, p=0.81, DHEA vs placebo respectively). Physical function measured by strength testing or bicycle $VO_2$ max assessments were unchanged.
**Conclusion:** This is the first study to show that DHEA replacement significantly increases insulin sensitivity in hypoadrenal subjects. These results may be significant in hypoadrenal populations where insulin treatment has been shown to improve morbidity and mortality, e. g. intensive care.This study also demonstrated significant reductions in total cholesterol, HDL, and triglycerides. This study also showed no demonstrable effect on skeletal muscle fractional synthesis rates or mitochondrial enzyme activity, thus suggesting possible explanations for the lack of effect of DHEA replacement on body composition, or skeletal muscle performance during exercise.
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**Glucocorticoid and insulin action on lipolysis in human subcutaneous and omental adipocytes**

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**Background and aims:** This in vitro study explores the metabolic differences between subcutaneous (sc) and omental fat depots with respect to in vitro effects of glucocorticoids, catecholamines and insulin.
**Materials and methods:** Adipocytes from sc and omental depots were obtained during abdominal surgery (cholecystectomy) in 21 non-diabetic subjects (F/M 15/6), age 15–87 yr, BMI 19–34 ($kg/m^2$). Cells were isolated with collagenase and metabolic studies were performed either directly after the biopsies or after a culture period of 24 h with or without dexamethasone (Dex, 0.3 μM). The cells were washed and basal and 8-bromo-cAMP (5 mM) stimulated lipolysis (glycerol release) as well as insulin's antilipolytic effect was assessed (60 min).
**Results:** Subcutaneous adipocytes had a 2-fold higher rate of basal as well as cAMP-stimulated lipolysis expressed per cell (p<0.01) (1.6-fold expressed/cell volume) as compared to omental adipocytes. The relative effect of insulin (0.1–100 μU/ml) to inhibit lipolysis did however not differ between the depots. Dex treatment for 24 h had no consistent effects on basal lipolysis rate in adipocytes from either depot but tended to increase the cAMP-stimulated lipolysis rate in both sc (p=0.07) and omental (p=0.09) adipocytes. Dex had no effects on the antilipolytic effect of insulin in either depot despite a marked reduction (by 50%, p<0.05) in the content of insulin signalling protein IRS-1 and PKB in omental but not sc adipocytes.
**Conclusion:** Surprisingly, freshly isolated human subcutaneous adipocytes displayed a higher rate of basal as well as cAMP-stimulated lipolysis as compared to omental adipocytes, but the effect of insulin to inhibit lipolysis did not differ between the depots. Furthermore, Dex treatment seems to elevate cAMP-stimulated lipolysis in adipocytes from both depots and this finding may be of relevance for the interaction between endogenous glucocorticoids and lipid metabolism in relation to the metabolic syndrome. Insulin's effect to inhibit lipolysis was however left intact despite down-regulation of insulin signalling proteins (IRS-1 and PKB) following Dex.

## 602

**Identification of glucocorticoid target genes in differentiated 3T3-L1 cells**

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**Background and aims:** Intracellular glucocorticoid levels in adipocytes are increasingly being recognized as an important aspect of the pathogenesis of insulin resistance and the metabolic syndrome. 11-β-HSD1 is an enzyme highly expressed in adipose tissue which regulates intracellular reactivation of glucocorticoids by converting cortisone into its active form, cortisol. Overexpression of 11-β-HSD1 in the adipose tissue of mice causes many of the phenotypes of metabolic syndrome, while a knockout protects mice from developing obesity and insulin resistance. While the action of glucocorticoids during adipocyte differentiation has been examined extensively, less is known about the effects of glucocorticoids on differentiated adipocytes. Here we examine gene expression profiles in differentiated 3T3-L1 adipocytes in response to glucocorticoids in the presence or absence of the non-specific 11-β-HSD1 inhibitor carbenoxolone.
**Materials and methods:** Differentiated 3T3-L1 adipocytes were transferred to serum-free medium for 3 hours and then treated with 1 uM cortisol, 1 uM cortisone, 100 nM dexamethasone, 20 uM carbenoxolone, cortisone + carbenoxolone, or a mock control for 2 h, 6 h, or 24 h. RNA was isolated and cRNA was generated for hybridization onto Affymetrix MOE430A arrays. Normalized data were filtered using ANOVA. Statistical significance was assigned as p<0.05.
**Results and conclusions:** We identified a common set of 455 genes, which are significantly regulated by dexamethasone, cortisone and cortisol. For 204 of these genes, regulation by cortisone was abolished by the 11-β-HSD1 inhibitor carbenoxolone, indicating dependence of the regulation on activation of cortisone to cortisol by 11-β-HSD1. Several distinct patterns of regulation were apparent by Eisen clustering. As expected, at early timepoints (2 h, 6 h) most regulated genes were induced by glucocorticoid treatment, likely representing direct transcriptional activation of these genes by the glucocorticoid receptor. In contrast, after 24 h of treatment, both induced and repressed gene sets were observed, possibly due to secondary effects mediated by the early response genes. We are particularly interested in a cluster of 45 genes, which are upregulated by glucocorticoids as early as two hours after treatment in a carbenoxolone-responsive manner. This cluster contains several previously described glucocorticoid-responsive genes as well several genes with a known function in insulin signaling, glucose or lipid metabolism. In addition, this cluster contains ~35 genes which have not been linked to glucocorticoid signaling or adipocyte function. We are currently following up on several candidate genes to further understand the mechanisms by which 11-β-HSD1 and glucocorticoids alter adipocyte function.

## 603

**Dexamethasone impairs insulin- but not contraction-stimulated glucose uptake**

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**Background and aims:** The glucocorticoid analogue dexamethasone is known to induce insulin resistance in skeletal muscles. So far, no data are

available concerning the effect of dexamethasone on insulin-stimulated PKB and GSK-3 phosphorylation. In addition to insulin, exercise is another important stimulus of glucose uptake in skeletal muscle. Whether dexamethasone impairs contraction-stimulated glucose uptake is unknown. The aims of the present study were to: 1) study the effect of dexamethasone on insulin-stimulated PKB and GSK-3 phosphorylation, and 2) determine whether contraction-stimulated glucose uptake is reduced in dexamethasone-treated rats.
**Materials and methods:** Male Wistar rats were treated with dexamethasone (1 mg/kg i.p.) for 13 days. Epitrochlearis (fast-twitch fibre) and soleus muscles (slow-twitch fibre) were dissected out and incubated in vitro in Krebs-Henseleit buffer. Muscle 2-[3H] deoxyglucose uptake was measured under basal condition, in the presence of maximal insulin concentration (10 mU/ml), and during electrical stimulation (200 ms/2s). Expression and phosphorylation of PKB and GSK-3 were measured by Western blot analysis and appropriated antibodies.
**Results:** Dexamethasone decreased insulin-stimulated glucose uptake in soleus and epitrochlearis (~40% and ~25%, respectively). This inhibition was accompanied by a decrease in insulin-stimulated PKB phosphorylation (~60% in soleus and ~50% in epitrochlearis). Dexamethasone reduced insulin-stimulated GSK-3α phosphorylation by ~20% and insulin-stimulated GSK-3β phosphorylation by ~30% in soleus. No inhibition was observed on tyrosine phosphorylation of the insulin receptor in dexamethasone-treated rats. Expression of PKBα, PKBβ, GSK-3α, and GSK-3β, were not reduced in muscles from dexamethasone-treated rats. Expression of p85α, however, was ~30% higher in dexamethasone-treated rats than in control rats. During electrical stimulation, glucose uptake was similar in dexamethasone-treated rats and control rats in both soleus and epitrochlearis muscles.
**Conclusion:** Dexamethasone treatment impairs insulin-stimulated glucose uptake by reducing insulin-stimulated phosphorylation of PKB and GSK-3 phosphorylation. Studies from transgenic mice have implicated that overexpression of p85α impairs insulin signalling. We suggest that elevated p85α impairs insulin-stimulated PKB activation in the intact skeletal muscles from dexamethasone-treated rats. Contraction-stimulated glucose uptake is not impaired by dexamethasone treatment, and the expression of the proteins involved in contraction-stimulated glucose uptake seems therefore not be regulated by dexamethasone.

# PS 45
# Pathophysiology in Type 2 diabetes

## 604

**Metabolic control analysis (MCA) of intravenous glucose tolerance in healthy humans**
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Metabolic Control Analysis (MCA) is a theoretical framework to assess the control of metabolic variables by components of the system (S) both in steady and in nonsteady states. MCA does so by computing the scaled coefficient of control (CC) of each component on the variable of interest (VOI), e. g. glucose (G) concentration. The CC quantifies the scaled fractional change (sign + or – if the change is in the same or in the opposite direction; typical range: –1 to +1) induced on the VOI by a minimal isolated change (conventionally +1%) in one of the other S components. MCA has never been applied to humans. We performed intravenous G tolerance (T) tests (IVGTT) in 10 (4 M, 6 F) subjects with normal G regulation (NGR) (Age: 25 ± 1.4 yrs; BMI: 21.5 ± 0.9 kg/m$^2$). The i.v. load was labeled with 6,6-H$^2$-G, to allow the distinction between endogenous (i. e. from endogenous G output (EGO)) and exogenous glycemia (i. e. from the load). We then analyzed the C-peptide, insulin (I), endogenous and exogenous G curves with published models, slightly modified for the present purpose, of I secretion (1$^{st}$ and 2$^{nd}$ phase (P) plus basal secretion) in response to G, and of I action both on G utilization (GU) and on EGO, plus quantification of G effectiveness on GU (GE), of glucosuria (Guria) and an estimate of brain/red blood cells GU (FGU). Thus, we built for each subject a global closed loop model of the G/I S, which reproduces the experimental IVGTT in silico and in which we could create isolated minimal changes in each S component and compute its CC on G level at 60 min during the IVGTT, as in our laboratory this glucose level best correlates with oral G T in both normal and altered G T states. The CCs of baseline glycemia, EGO, I-mediated GU, GE, FGU and Guria were +0.22 ± 0.04, +0.14 ± 0.03, –0.33 ± 0.08, –0.19 ± 0.04, –0.17 ± 0.02 and –0.009 ± 0.004, respectively. Remarkably, the CC of the amount of the i.v. G load was –0.04 ± 0.12 (not statistically different from 0). The CC of total I action (i. e. on both GU and on EGO) was not statistically different from global beta-cell function (–0.48 ± 0.10 vs –0.59 ± 0.13, p=NS). The individual CC of 1$^{st}$ and 2$^{nd}$ P were very similar similar (–0.22 ± 0.07 and –0.24 ± 0.04, p=NS). Thus, applying MCA for the first time in humans shows that in NGR: 1. the I/G S is poised to offset completely any influence of the size of an i.v. load on G T; 2. I action and beta-cell function may play quantitatively similar roles in determining G T; 3. 1$^{st}$ and 2$^{nd}$ P I secretion are equally important in controlling G T.

## 605

**Visceral fat accumulation in nondiabetic subjects is associated with reduced insulin sensitivity but normal beta-cell function**
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**Background and aims:** Preferential visceral adipose tissue (VAT) deposition has been associated with the presence of insulin resistance which is chraracterized by increased insulin secretion. The impact of VAT accumulation on beta-cell function is on the other hand not clear as it has been confounded by the presence of obesity and glucose intolerance.
**Materials and methods:** We measured VAT and subcutaneous (SAT) fat depots (kg) by multislice MRI in 62 nondiabetic subjects (age=24–70 yrs) ranging in BMI from 21 to 39 kg · m$^{-2}$ and in VAT/SAT ratio from 0.04 to 0.87. 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). Insulin secretion was evaluated by deconvolution of C-peptide data; dynamic indices of beta-cell function (glucose sensitivity, rate sensitivity, and potentiation) were derived from model analysis of the insulin secretory response to OGTT.
**Results:** Independently of or in interaction with obesity, preferential visceral fat accumulation was associated with prevalence of male gender, older age, larger waist circumference, and larger fat depots in both the abdominal

visceral region but also in the subcutaneous volume of the abdomen, particularly in the deeper strata. After adjusting for gender, age, and BMI, both VAT and SAT were independently associated with impaired insulin sensitivity (partial *r*'s of -0.27 and -0.36, $p<0.05$ and $p<0.01$, respectively) and increased insulin secretion (partial *r*'s of 0.27 and 0.26, $p<0.05$ for both). Consequently, the VAT/SAT ratio was unrelated to insulin sensitivity or response. None of the dynamic indices of beta-cell function was associated with either VAT, SAT, or their ratio.
**Conclusion:** When accounting for the main determinants of regional fat distribution (gender, age, and obesity), fat accumulation in either VAT or SAT is associated with insulin resistance and compensatory insulin hypersecretion in nondiabetic subjects. The relative distribution of abdominal fat (VAT/SAT) is by itself neutral to both insulin action and beta-cell function.

## 606

**Endogenous glucose production under basal and postprandial conditions in Type 2 diabetes**
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**Background and aims:** In subjects with type 2 diabetes, fasting glycemia and the underlying production of glucose both decrease continuously during continued fasting over the course of the day. This study was designed to reassess the suppression of endogenous glucose production (EGP) against this time-varying background.
**Materials and methods:** Subjects with (i) recently (<6 mos) diagnosed type 2 diabetes (<6 mos., n=6, 99 ± 7 kg, NEW), (ii) more established diabetes (5.0 ± 0.9y, n=8, 92 ± 5 kg, EST) and (iii) weight-matched controls (n=5, 89 ± 9 kg, CONT), each underwent two studies, following a 12–14 h overnight fast: (a) a 10 h infusion of [6-$^3$H]glucose and (b) a 10 h infusion of [U-$^{13}$C]glucose combined with a mixed meal at 4 h, in which the carbohydrate moiety was glucose, labelled with [1-$^{14}$C]glucose. Glucose kinetics were assessed using the sampled tracers and glucose, and a compartmental model of appropriate order. Constant model parameters were estimated individually for each study.
**Results:** During the fasting study (a), glucose concentrations were 7.9 ± 0.4, 11.6 ± 0.8 and 5.2 ± 0.2 mM at t=0, 6.7 ± 0.3, 10.2 ± 0.6 and 5.2 ± 0.3 mM at t=4 h, and fell further to 6.2 ± 0.4, 9.5 ± 0.4 and 5.0 ± 0.3 mM at t=6 h in NEW,EST and CONT respectively. During the meal study (b), the conc. were 7.5 ± 0.3, 11.9 ± 0.6 and 5.0 ± 0.2 mM at t=0, 6.6 ± 0.4, 10.6 ± 0.9 and 5.2 ± 0.2 mM and 11.7 ± 1.4, 17.0 ± 1.8 and 7.3 ± 0.6 mM at t=6 h (2 h postprandially) in NEW, EST and CONT. The metabolic clearance rate of glucose remained near constant during the fasting study at 1.4 ± 0.1, 1.1 ± 0.06 and 1.6 ± 0.08 μmol · kg$^{-1}$ · min$^{-1}$ in NEW, EST and CONT. During the meal study, MCR increased to 2.8 ± 0.4, 1.6 ± 0.2 and 4.5 ± 0.5 μmol· kg$^{-1}$ · min$^{-1}$ at t=6 h (2 h postprandially) in the respective groups. Endogenous glucose production, on the other hand, decreased during fasting: from 12.8 ± 0.9, 14.5 ± 1.2 and 8.8 ± 1.0 μmol· kg$^{-1}$ · min$^{-1}$ at t=0 to 9.8 ± 1.0, 11.8 ± 1.2 and 8.5 ± 0.7 μmol · kg$^{-1}$ · min$^{-1}$ at t=6 h in NEW, EST and CONT. During the meal study, EGP fell from 13.1 ± 1.0, 15.0 ± 0.9 and 8.5 ± 0.7 μmol · kg$^{-1}$ · min$^{-1}$ to 11.2 ± 1.0, 13.2 ± 1.1 and 8.5 ± 0.5 μmol · kg$^{-1}$ · min$^{-1}$ at t=4 h to 8.8 ± 0.8, 10.9 ± 0.9 and 3.3 ± 0.9 μmol · kg$^{-1}$ · min$^{-1}$ at 6 h (2 h postprandially, $p<0.05$, NEW + EST vs CONT). Overall, for the entire absorption period, EGP was suppressed by an average of 47 ± 10%, 45 ± 7% and 53 ± 8% in NEW, EST and CONT when compared to the average preprandial glucose production. However, EGP was suppressed by 19 ± 5%, 17 ± 5% and 56 ± 7%, *when it was compared to the rates of production which would have prevailed without the meal* ($p<0.05$, NEW + EST vs CONT).
**Conclusion:** A major part of the postprandial decline in EGP can therefore be attributed to a continuation of the daytime fall in this rate. It appears that diurnal factors which control EGP in type 2 diabetes, override the meal-related regulation leading to a virtual decoupling of the basal pattern of EGP from meal-associated regulatory factors, such as insulin and absorptive glucose. This appears to occur early in the course of the disease.
*Supported by: Canadian Diabetes Association*

## 607

**Impaired insulin sensitivity of hepatic glycogen metabolism in Type 2 diabetes**
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**Background and aims:** Type 2 diabetic humans (T2DM) exhibit reduction in both hepatic glycogen storage and suppression of endogenous glucose production (EGP) after meal ingestion. These defects of postprandial hepatic glucose metabolism could result from impaired insulin secretion leading to a decreased portal vein insulin:glucagon ratio and/or from increased availability of plasma free fatty acids (FFA). Thus we aimed to examine whether hepatic glycogen storage and suppression of EGP could be restored in T2DM under conditions simulating the physiological postprandial rise in portal vein insulin:glucagon ratio and fall of plasma FFA.
**Materials and methods:** Hyperinsulinemic (40 mU/m$^2$ · min)-hyperglycemic (180 mg/dl)-somatostatin-clamps (300 min) were performed in 6 T2DM (55 ± 4 a; BMI: 28 ± 1 kg/m$^2$; HbA1C: 7.4 ± 0.1%) and 6 CON (53 ± 4 a; BMI 26 ± 1 kg/m$^2$; HbA1C: 5.4 ± 0.1%). EGP was determined with [6,6-$^2$H$_2$]glucose infusion. Hepatic glycogen concentrations were measured with in vivo $^{13}$C nuclear magnetic resonance spectroscopy ($^{13}$C NMRS). Fluxes through hepatic glycogen synthase and phosphorylase were assessed during [1-$^{13}$C]/[1-$^{12}$C]glucose infusion (pulse-chase method). Pathways of hepatic glycogen synthesis (direct vs. indirect) were obtained by the acetaminophen glucuronide probe technique.
**Results:** During the clamp tests (30–300 min), plasma concentrations of glucose (T2DM: 181 ± 2 vs. CON: 178 ± 2 mg/dl), insulin (68 ± 1 vs. 69 ± 2 μU/ml), glucagon (64 ± 2 vs. 61 ± 3 pg/ml) and FFA (55 ± 8 vs. 52 ± 12 μmol/l) were identical in both groups. Nevertheless, rates of EGP during the clamp (30–300 min) were higher in T2DM (0.53 ± 0.05 vs. CON: 0.04 ± 0.04 mg·kg$^{-1}$ · min$^{-1}$, $p<0.02$). Hepatic glycogen concentrations increased linearly in both groups during the clamp but flux through hepatic glycogen synthase was ~46% lower in T2DM (0.63 ± 0.12 vs. CON: 1.17 ± 0.15 mg·kg$^{-1}$ · min$^{-1}$, $p<0.03$) with similar contribution of the direct pathway in both groups (T2DM: 60 ± 10% vs. CON: 65 ± 2%, p= 0.416). The simultaneous flux through hepatic glycogen phosphorylase was not different between the groups (T2DM: 0.21 ± 0.07 vs. CON: 0.23 ± 0.11 mg · kg$^{-1}$ · min$^{-1}$, ns) resulting in ~54% lower rates of net hepatic glycogen synthesis in T2DM (0.42 ± 0.10 vs. CON: 0.91 ± 0.16 mg · kg$^{-1}$ · min$^{-1}$, $p<0.03$). Relative hepatic glycogen turnover was slightly but not significantly increased in T2DM (31 ± 11% vs. CON: 19 ± 11%, p= 0.460).
**Conclusion:** In T2DM hepatic glycogen storage and suppression of EGP are not normalized under standardized conditions of postprandial like portal vein insulin:glucagon ratio and decreased availability of FFA. Thus, liver glucose metabolism of T2DM is less sensitive to the action of insulin.

## 608

**The effect of vitamin $D_3$ on insulin secretion and peripheral insulin sensitivity in patients with Type 2 diabetes (T2D)**
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**Background and aims:** The combined effects of insulin resistance and defective insulin secretion in the pathogenesis of T2D are well established. Over the past 30 years several studies in humans have also demonstrated that vitamin D deficiency causes reduced insulin secretion and that 1,25(OH)$_2$D$_3$ improves glucose intolerance and insulin resistance. The aim of this study was to evaluate the effect of vitamin $D_3$ supplementation on insulin secretion and insulin resistance in T2D patients.
**Materials and methods:** Eight male (Group A, mean age 48.9 ± 1.46 years, mean BMI 27.93 ± 0.31) and 7 female patients (Group B, mean age 52.71 ± 1.74 years, mean BMI 28.9 ± 0.36) with T2D of recent onset (less than 5 years) were evaluated. The first (FPIS) and second (SPIS) phases of insulin secretion were studied during FSIVGTT. Peripheral insulin resistance was estimated with the Minimal Model (MinMod 3.0). The patients were treated with alphacacidol [1$_a$(OH)vitamin D$_3$] 1 μg daily for 3 months.
**Results:** In Group A, plasma 25(OH)D$_3$ values were at the normal range at baseline [mean plasma 25(OH)D$_3$ 29.2 ± 3.79 ng/ml, normal (nl): 10–50] and increased to 46.08 +/–3.72 at the end of the study. All patients were normocalcemic (9.04 ± 0.1 mg/dl). The integrated AUC for insulin after the glucose load did not show any significant change at the end of the study, but the FPIS (8 minutes) increased significantly from 196.81 ± 8.49 at baseline to 261.7 ± 8.43 ($p<0.003$). There was a reduction in the index of insulin

sensitivity (Si) that reached statistical significance (2.025 ± 0.481 at baseline, 1.679 ± 0.426 at three months, $p<0.03$). When metabolic parameters were evaluated, at the end of the study, there was no statistical difference in the values for HbA1 c, serum triglycerides, HDL and LDL-cholesterol levels. In Group B, plasma 25(OH)$D_3$ values were at the low normal range at baseline [mean plasma 25(OH)$D_3$ 11.67 ± 1.84 ng/ml] and rised to 30.23 ± 2.15 ng/ml at the end of the study. The integrated AUC for insulin (FPIS + SPIS) after the glucose load decreased significantly [7958.38 ± 1532.15 at baseline, 7558.79 ± 1626 ($p<0.03$) at the end of the study], but the FPIS (8 minutes) increased significantly from 266.06 ± 27.01 at baseline to 310.39 ± 30.98 ($p<0.0003$). The AUC for glucose decreased from 29258.36 ± 3438.43 at baseline to 25370.93 ± 2644.4 ($p<0.01$). There was a statistically significant increase in Si (1.4848 ± 0.55 at baseline, 2.8276 ± 1.17 at three months, $p<0.05$). HbA1 c decreased from 6.69 ± 0.12 to 6.46 ± 0.1 ($p<0.05$). At the end of the study, serum triglycerides decreased from 153.86 ± 7.5 to 143 ± 8.4 ($p<0.05$) but there was no significant change in HDL and LDL-cholesterol levels.
**Conclusion:** Considering that reduced FPIS and insulin resistance are major metabolic defects in early T2D and the favourable effect of alpha-cacidol in female postmenopausal patients, it may be possible that vitamin $D_3$ defficiency may contribute to the impairement of insulin secretion and probably insulin action. Our results suggest that vitamin $D_3$ supplementation could be of value in the early course of T2D.

## 609

**Evaluation of pancreatic exocrine function in Type 1 and Type 2 diabetes mellitus by secretin test**

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**Background and aims:** Exocrine pancreas and endocrine islets of Langerhans are closely interrelated with each other and pancreatic exocrine dysfunction is found in some of the patients with diabetes mellitus. However, pathophysiological difference of exocrine dysfunction between type 1 and type 2 diabetes mellitus is not understood. In this study, we evaluated pancreatic exocrine function of patients with type 1 and type 2 diabetes mellitus by secretin test, the most reliable pancreatic function test.
**Materials and methods:** 5 patients with type 1 diabetes mellitus and 22 patients with type 2 diabetes mellitus were included in this study. In secretin testing, a Bartelheimer's double-balloon tube was used. After intravenous administration of 100 CHRU/body of secretin (Eisai, Tokyo, Japan), duodenal juice was aspired into and collected in 10-min samples for 1 hour. The total volume (V), total amylase output (AO), and maximal bicarbonate concentration (MBC) were measured. The lower limit of the parameters was set according to the data obtained from the 22 normal controls. The mean ± SD of V, AO, and MBC in normal controls were 251 ± 68 ml/h, 146,000 ± 47,000 U/h, and 94 ± 7 mEq/L, respectively. Functional scoring was as follow; severe = MBC below M - 2SD plus both V and AO below M - SD; moderate = MBC below M - 2SD plus V or AO below M - SD; mild = MBC below M - 2SD or both V and AO below M - SD; borderline = V or AO below M - SD; normal = all normal.
**Results:** In 5 patients with type 1 diabetes mellitus, 2 patients (40%) had severe exocrine dysfunction, compatible to definite chronic pancreatitis. 2 patients (40%) had mild dysfunction and 1 patient (20%) had borderline dysfunction. In the 2 patients of severe exocrine dysfunction, the hyperglycemia was poorly responsive to treatment with insulin and blood levels of HbA1C were above 8%. Pancreatic exocrine dysfunction was found in 50% of the patients with type 2 diabetes mellitus; 1 patient (5%) had severe, 2 patients (9%) had moderate, and 8 patients (36%) had mild exocrine dysfunction. In type 1 diabetes mellitus, V, AO, and MBC were 143 ± 32 ml/h, 63,000 ± 28,000 U/h, and 90.6 ± 24.6 mEq/L. In type 2 diabetes mellitus, V, AO, and MBC were 217 ± 70 ml/h, 129,000 ± 53,000 U/h, and 84.5 ± 13.8 mEq/L. V and AO were significantly lower in the patients with type 1 diabetes mellitus than in the patients of type 2 diabetes mellitus ($p< 0.05$).
**Conclusion:** Pancreatic exocrine dysfunction was found in most of the patients with type 1 diabetes mellitus. It was more serious than in type 2 diabetes mellitus and was related with blood levels of HbA1C. Exocrine dysfunction was found in half of the patients with type 2 diabetes mellitus. These data suggest that pancreatic exocrine dysfunction in diabetes mellitus is caused by both hyperglycemia and decrease of insulin secretion.

# PS 46

# Metabolic syndrome: insulin resistance

## 610

**A calculator for HOMA**

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**Background and aims:** The HOmeostasis Model Assessment (HOMA) is a mathematical model which can estimate an individual's degree of insulin sensitivity (HOMA %S) and level of beta cell function (HOMA %B) from simultaneous measurements of fasting plasma glucose (FPG) and fasting plasma insulin concentrations. HOMA models the physiologic glucose/insulin feedback system mathematically. It incorporates data on pancreatic beta-cell function plus peripheral (muscle and brain) and hepatic insulin sensitivity as well as glucose and insulin measured in the fasting state. The model estimates an individual's insulin sensitivity based on the assumption that any one combination of glucose and insulin is associated with a given insulin sensitivity, or, conversely, their insulin resistance. Its simplicity, reproducibility and correspondence to glucose clamp derived estimates of insulin resistance and stimulatory test estimates of insulin secretion have meant that HOMA has become a much used method for estimating insulin sensitivity and beta cell function in people with non-insulin treated Type 2 diabetes, particularly in large-scale studies. An updated HOMA model has been developed (version 2) which has been adapted to work with modern insulin assays. Although linear equations are available which can give approximate HOMA %S and %B estimates, the most accurate results are obtained using the HOMA model in its computerised form.
**Materials and methods:** The Fortran computer program for HOMA version 2 has been converted to C and optimised for speed of calculation. An Applications Programming Interface (API), encapsulating the HOMA software as an ActiveX module or as a Macintosh shared library, has been developed to facilitate incorporation of the program into other software packages.
**Results:** The updated software was checked for validity by checking the results obtained against those from the existing computer model. The new API was used with bespoke interfaces to enable the program to run on a variety of computer platforms as a stand-alone application to permit rapid calculation of an individual's HOMA %S and %B. The API was incorporated also within a Microsoft Excel Spreadsheet to facilitate the calculation of %S and %B values for many individuals simultaneously
**Conclusion:** This software implementation of HOMA version 2 can calculate %S and %B values instantly from paired FPG and FPI values (or fasting specific insulin or C-peptide). Its availability as free download for a variety of computer platforms (www.dtu.ox.ac.uk/homa) should assist clinicians and researchers to derive better estimates of %S and %B than can be obtained from the linear approximations in common use.

## 611

**Cut points for insulin sensitivity indices in normal and prediabetic subjects**

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**Background and aims:** Demanding quantification of insulin sensitivity using euglycemic clamp has led to invention of a large number of more simple approaches. For majority of the insulin sensitivity indices (ISI) utilising either fasting or OGTT-derived glycemia and insulinemia, very limited information on the relevant reference values to distinguish between insulin sensitive and resistant subjects, using rather small cohorts, has been published so far. Therefore, the aim of this study was an attempt to define the relevant cut points for insulin resistance (IR) and the ranges of the most frequently obtained values for selected ISI as HOMA (an index of hepatal IR), ISI Cederholm (an index of peripheral insulin sensitivity) and ISI Matsuda (an index of whole-body insulin sensitivity) in normal and prediabetic Slovak population.
**Materials and methods:** A total of 1054 subjects (401 men and 653 women) with no previous evidence of diabetes or other dysglycemias, aged 45 ± 0.3 (mean ± SEM) and mean BMI 26.7 ± 0.1 kg/m$^2$ were studied. A standard 75-g OGTT was carried out in each subject. Subjects with normal glucose

homeostasis, or prediabetes, i.e. those with IFG, IGT, or their combination, were also divided according their BMI into 3 groups: normal weight, overweight, and obesity. Selected ISI were calculated from plasma glucose (glucose oxidase method, Hitachi analyser) and insulin levels (electrochemiluminescence, Roche) as obtained before and 60 and 120 min after the oral glucose load. The cut point value for IR was estimated as the 75$^{th}$ percentile of HOMA in the group with normal glucose homeostasis, or as the 25$^{th}$ percentile of ISI Cederholm and ISI Matsuda in the same group (based upon the WHO definition, where IR is defined as the highest quartile of the HOMA in subjects with no metabolic abnormalities).
**Results** are summarised in Table 1. Cut point for HOMA was 1.55, thus all subjects with higher values may be considered as insulin-resistant. Cut points for ISI Matsuda and ISI Cederholm were 7.1 and 72, respectively, thus all subjects with lower values may be considered as insulin-resistant. Furthermore, the most frequently obtained values of selected indices (estimated as the 25$^{th}$–75$^{th}$ percentile) were introduced, in order to allow the comparison of obtained IR value within a group of subjects defined by the same stage of glucose homeostasis (Table 1).
**Conclusion:** a) using a large randomly selected group of clinically healthy people, we have proposed cut points for selected insulin sensitivity indices: i.e. for HOMA, Matsuda and Cederholm; b) furthermore, ranges of the most frequently obtained values of ISI were defined for the individual categories of dysglycemia; c) these results might provide a better insight in identification of IR in prediabetic subjects without clinical symptoms of a disease.

ISI expressed as median (25–75%), #p < 0.01, *p < 0.001 vs. normal, cut points-bold

| | **normal glucose homeostasis** (n=521) | **isolated IFG** (n=411) | **isolated IGT** (n=37) | **IFG+IGT** (n=85) |
|---|---|---|---|---|
| **IR HOMA** | 1.10 (0.74–**1.55**) | 2.00 (1.34–2.80)* | 1.39 (1.08–2.22)# | 2.44 (1.66–3.67)* |
| **ISI Cederholm** | 94 (**72–120**) | 70 (56–87)* | 47 (40–52)* | 40 (35–48)* |
| **ISI Matsuda** | 10.8 (**7.1–16.4**) | 6.1 (4.3–9.2)* | 5.8 (4.0–8.2)* | 3.8 (2.5–5.6)* |

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## 612

**The updated HOMA model: comparison of performance against euglycaemic and hyperglycaemic clamps**
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**Background and aims:** Homeostatic model assessment (HOMA) is used to determine insulin sensitivity and beta-cell function from fasting plasma glucose and insulin concentrations. The model has recently been updated: the presence of proinsulin has been taken into account in the radioimmunoassay insulin model, renal glucose losses have been included in the model and the insulin secretion curve has been modified to allow for an increase in insulin secretion in response to hyperglycaemia.The aims are: (1) to compare estimates of insulin sensitivity (%S) and beta-cell function (%B) derived from the updated HOMA model (HOMA2) with estimates from euglycaemic and hyperglycaemic clamps. (2) To examine the effect of using 3 plasma samples at 5 minute intervals versus using a single sample on intra-subject reproducibility.
**Materials and methods:** 30 subjects with diet-controlled type 2 diabetes underwent basal sampling for HOMA followed by a hyperglycaemic clamp and hyperinsulinaemic euglycaemic clamp.
**Results:** Subject characteristics: 24 male, 8 female, median duration of diabetes 2.54 (IQR: 0.9–3.8) years. Mean (SD): age 61.8 (7.7) years, BMI 29.5 (4.0) Kg/m$^2$, HbA1c 7.6 (0.9)%, fasting glucose 7.6 (1.5) mmol/l; adiponectin 24.5 (11.3) ng/ml, M/I 26.8 (12.1) L/kg/min. Geometric mean (SD range): FPI 93.5 (54.2–161.4) pmol/l, HOMA%B 64.7 (41.2–101.7)%, HOMA%S 52.9 (30.7–91.1)%. There was a strong correlation between HOMA2%B and the steady state insulin secretion during the final 30 minutes of the hyperglycaemic clamp following logarithmic transformation ($r=0.92$, $p<0.0001$); and between Log HOMA%S and log M/I derived from the euglycaemic ($r=0.82$, $p<0.0001$). There was a similar degree of correlation between adiponectin and M/I ($r=0.52$, $p=0.0046$) and adiponectin and HOMA-%S ($r=0.53$, $p=0.0032$). Data show near perfect correlations between HOMA%B and HOMA%S computed from the mean of three basal samples at 5 minute intervals and from a single basal sample ($r=0.99$, $p<0.0001$). However the use of a single sample gives intra-subject CVs of 10.3% for HOMA%S and 7.6% for HOMA%B compared to 5.8% and 4.3% respectively when the mean of three samples is used.
**Conclusion:** Estimates derived from the HOMA2 model correlate well with estimates from clamps. Perfect correlations would not be expected since HOMA yields basal estimates whereas clamps give estimates at the maximally stimulated end of the dose response curve. The use of single sample yields similar results as the mean of 3 samples, although the intra-subject CV improves with 3 samples.

## 613

**Females are intrinsically more insulin resistant than males, but males are at greater risk of Type 2 diabetes**
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**Background and aims:** In childhood, type 2 diabetes (T2D) affects females more than males. In adulthood, the reverse is true – males are more prone. This switch in gender dominance deserves explanation because it may help to explain the pathogenesis of T2D. When considering the possibilities, it is important to distinguish susceptibility (intrinsic/genetic) from risk (acquired/environmental), because their contributions may vary at different times of life. The aim of this study was to explore the relative contributions of genes and environment to insulin resistance in childhood and adulthood. We hypothesised that females are intrinsically more insulin resistant than males, but that males acquire insulin resistance in early adulthood.
**Materials and methods:** Measurements of height, weight (BMI), sum of 5 skin-folds, physical activity (CSA accelerometer), waist circumference (WC), resting metabolic rate by gas exchange (RMR) and insulin resistance (HOMA-IR) were made in young children from the EarlyBird study. These children had been randomly selected from 34 primary schools in the city of Plymouth, UK. There were 137 girls and 170 boys, mean age 4.9 ±SD 0.2y, at the time of this report. BMI, WC and HOMA-IR were also recorded in their parents (298 mothers, mean age 33.4y and 250 fathers, mean age 36.4y). The genders were compared for HOMA-IR before and after adjustment for WC.
**Results:** The girls were 33% more insulin resistant than the boys, even after adjustment for all anthropometric, physical activity and RMR differences ($p<0.001$). The fathers were 7% more insulin resistant than the mothers – until adjustment for WC. Adjustment reversed the switch, making the mothers 25% more insulin resistant than the fathers. Importantly, the regressions relating log HOMA-IR to WC in the mothers and fathers were identical in gradient (both 0.027, $p<0.001$), but significantly displaced suggesting, once again, that females are intrinsically more insulin resistant than males.
**Conclusions:** These simple observations may have more fundamental implications. WC is a proxy for visceral fat mass, which is thought to underlie insulin resistance. Children at 5y have similar WC, while adult males acquire a higher WC than females. Indeed, the greater WC of adult males completely explains the gender change in insulin resistance from childhood to adulthood. The intrinsically higher insulin resistance of females throughout life and acquisition of insulin resistance by adult males would together predict the observed higher prevalence of T2D among females in childhood and among males in adulthood. They would also predict a higher prevalence of T2D among adult females once adjustment was made for body fat, and this is observed clinically. Finally, birth weight is dependent on insulin action, and the intrinsically higher insulin resistance of females may explain their universally lower birth weight (the 'gender insulin hypothesis'). Focus on the prevention of weight gain in young males could have a major impact on the overall incidence of T2D.
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## 614

**Thyrotropin is associated with the insulin resistance syndrome in an euthyroid population**
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**Background and aims:** Overt hypothyreoidism is associated with cardiovascular disease, but it is controversial whether there is a similar association between subclinical hypothyreoidism and cardiovascular disease. The association between cardiovascular disease and the insulin resistance

syndrome is strong. The association of thyroid function with the insulin resistance syndrome is based on a few studies with a small number of study subjects. The aim of this study was to assess the association of THS with different components of the insulin resistance syndrome in an euthyroid population.
**Materials and methods:** The study population consisted of 63-year-old euthyroid persons (serum thyrotropin (TSH) concentration 0.3–5.0 who were not on thyroid substitution or antithyroid medication, n=521, 223 men) in the City of Oulu in Northern Finland. Partial correlation coefficients between TSH and different variables of the insulin resistance syndrome adjusted for sex were calculated.
**Results:** Women had higher concentrations of serum TSH compared to men (2.33 vs. 2.10, p=0.007). The serum TSH concentration was positively associated with the waist-to-hip circumference ratio (partial correlation coefficient adjusted for sex, r=0.106, p=0.021), the 2-hour glucose concentration during a 75-g oral glucose tolerance test (OGTT) (r = 0.130, p=0.005), logarithms of fasting and 2-hour serum insulin concentrations during the OGTT (r=0.152, p=0.001 and r=0.156, p= 0.001, respectively), serum fasting triglycerides (r=0.099, p=0.025) and serum uric acid (r=0.123, p=0.007) and negatively associated with the quantitative insulin check index (r=–0.146, p=0.002). The serum TSH concentration did not correlate with waist circumference, body mass index, fasting serum glucose concentration, other lipids, urine albumin-to-creatinine ratio and blood pressure.
**Conclusion:** Thyroid function is associated with most components of the insulin resistance syndrome.

## 615

**Serum gamma-glutaryltransferase concentration within its normal range is closely related to the presence of diabetes and cardiovascular risk factors**

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**Background and aims:** Although many studies reported the association between serum gamma-glutamyltransferase (GGT) and cardiovascular risk factors, there have been few reports about this association within normal range of GGT.
**Materials and methods:** Medical records of 33,248 subjects who visited health promotion center to have physical check-up between 2001 and 2003, were investigated. Subjects with hepatic enzyme/GGT concentrations higher than three times the upper limit of the reference range and/or positve test for hepatitis C virus antibody and/or positive test for hepatitis B virus surface antigen and/or history of current antidiabetic/antihypertensive/antilipid medications and/or white blood cell (WBC) count higher than 10,000 cells/ml, were excluded. Total subjects were classified into five groups according to their serum GGT concentrations [quartiles of normal range of GGT (group 1, 1≤ GGT ≤13, n=6958; group 2, 14≤ GGT ≤19, n=7416; group 3, 20≤ GGT ≤28, n=6874; group 4, 29≤ GGT ≤50, n=7123) and elevated GGT (group5, 51≤ GGT ≤150, n=4867)].
**Results:** With increase of GGT, frequencies of diabetes and impaired fasting glucose (IFG) increased (diabetes 0.4, 0.9, 2.3, 3.7, 5.5%; IFG 1.2, 2.9, 4.3, 7.5, 10.5% in each group). And also, frequencies of hypertension, obesity (body mass index ≥ 25 kg/m$^2$) and dyslipidemia (LDL-cholesterol ≥ 4.1 mmol/l and/or triglyceride ≥ 2.46 mmol/l and/or HDL-cholesterol < 1.16 mmol/l) increased, with increase of GGT (hypertension, 6.1, 8.8, 10.1, 11.6, 14.8%; obesity, 12.3, 20.5, 38.2, 55.8, 72.9%; dyslipidemia, 26.1, 38.8, 50.9, 61.8, 70.0%). Moreover, GGT concentrations within its normal range were related the presence of diabetes/IFG, hypertension, obesity and dyslipidemia, after adjustment of age, gender, smoking, alcohol consumption, family history of diabetes, WBC counts, serum alanine aminotransferase and serum aspartate aminotransferase. Odds ratios (95% CI) in group 4 (highest quartile of normal range of GGT), with group 1 (lowest quartile of normal range of GGT) as the referent group, were 5.58 (3.57–8.72) for diabetes, 4.07 (3.13–5.30) for IFG, 1.21 (1.04–1.40) for hypertension, 2.30 (2.07–2.56) for obesity and 2.45 (2.19–2.73) for dyslipidemia.
**Conclusion:** Our data showed that even within its normal range, serum GGT concentrations were closely associated with the presence of diabetes and cardiovascular risk factors.

## 616

**Which features of the metabolic syndrome predict fatty liver in obese men?**

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**Background and aims:** It has recently been recognised that nonalcoholic steatohepatitis (NASH) can affect individuals with the metabolic syndrome as a consequence of fatty liver, but an explanation for the association is lacking. As NASH is a cause of liver fibrosis it is important to understand the pathogenesis.
**Materials and methods:** We have studied 31 men aged 40–65 with a wide range of BMI to determine which features of the metabolic syndrome predict fatty liver determined by ultrasonography. Obesity was assessed by BMI, anthropometry, DEXA, bioimpedance, air-displacement plethysmography and abdominal MRI. Insulin sensitivity was determined by glucose disposal during a euglycaemic hyperinsulinaemic clamp. Non-esterified fatty acid (NEFA) suppression during the OGTT was measured because NEFA suppression is linked to insulin sensitivity in fat and increased NEFA levels cause hepatic triglyceride accumulation. Adiponectin levels were also measured as a marker of adipocyte function.
**Results:** Features of the metabolic syndrome were compared between obese subjects with and without fatty liver and compared with lean subjects using analysis of variance. There was no significant difference in fasting triglyceride, fasting HDL cholesterol, blood pressure or adiponectin between obese subjects with and without fatty liver.
Subjects with fatty liver had slightly more total fat (36% v 33% $p < 0.05$) but no more visceral fat compared with subjects without fatty liver. Subjects with fatty liver were significantly more insulin resistant (glucose disposal 4.4 min · mg · kg$^{-1}$ v 6.7 min · mg · kg$^{-1}$ $p<0.05$) than subjects without fatty liver. Subjects with fatty liver had significantly impaired NEFA suppression (419 hr · nmol · l$^{-1}$ v 272 hr · nmol · l$^{-1}$ $p<0.05$) compared with subjects without fatty liver. Both insulin resistance and area under the NEFA curve predicted the presence of fatty liver ($p<0.05$).
**Conclusion:** The most important predictors of fatty liver are insulin resistance and area under the NEFA curve.
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## 617

**Influence of fat distribution and prenatal environment on insulin sensitivity**

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**Background and aims:** Adverse intrauterine environment is associated to a defect in glucose tolerance and type 2 diabetes later in life. A relation is seen between low birth weight and obesity. Obesity is a risk factor for development of type 2 diabetes. To determine the impact of fat distribution and prenatal environment on in vivo insulin action in twins.
**Materials and methods:** A total of 108 monozygotic (MZ) and 88 dizygotic (DZ) young (25–32 years) and elderly (58–66 years) twins underwent a hyperinsulinaemic euglycaemic clamp (to determine insulin action, Rd) and a DXA-scanning (to measure fat distribution). The prenatal environment was estimated by birth weight (BW) and zygosity status. In order to examine the associations between Rd (dependent variable) and BW, zygosity and fat distribution (explanatory variables) a multiple regression analysis was developed allowing for adjustment according to zygosity status.
**Results:** Rd was explained by the interactions between lower body fat percent and zygosity ($p = 0.04$), BW and truncal fat percent ($p = 0.04$), truncal fat percent and lower body fat percent ($p < 0.0001$) and BW and truncal-lower body fat percent ($p = 0.032$). Lower body fat percent contributed positively to Rd, and the increase in Rd with increasing lower body fat percent was significantly higher for DZ as compared to MZ twins. A negative influence on Rd with increasing trunk fat % was demonstrated. In the low end of the scale for trunk fat %, twins with the lowest birth weight had the highest Rd. Increasing lower body fat percent reduced the negative impact of truncal fat percent on Rd in both MZ and DZ twins. The ratio between truncal-lower body fat percent influenced Rd negatively but this effect diminished with increasing BW.
**Conclusion:** Zygosity, birth weight and fat distribution play significant roles for insulin sensitivity in twins. Being DZ twin is more favourable

compared to MZ twin in relation to insulin sensitivity. Low birth weight is associated with decreased insulin sensitivity. Truncal fat percent is negatively associated to insulin sensitivity, while lower body fat percent seems to play a protective role.

## 618

**Association between adiponectin, energy expenditure (EE) and lipid metabolism in subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and Type 2 diabetes mellitus (DM)**

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**Background and aims:** Adiponectin, secreted by fat cells, is a marker of insulin resistance and has potential regulatory functions in energy metabolism. The aim of the study was to evaluate the relationships between adiponectin, insulin resistance, EE and lipid metabolism in NGT, IGT and DM.
**Materials and methods:** We evaluated 60 subjects with different phases of glucose tolerance (NGT, IGT and newly diagnosed DM). They were classified based on the results of the oral glucose tolerance test (75 g). At these patients, adiponectin (quantitative human adiponectin/AICRP 30), insulin (quantitative measurement with immunoenzymetric assay) and parameters of lipid metabolism (TG and HDL-Chol) were determined. Data of energy metabolism were analysed by indirect calorimetry (DELTATRAK metabolic monitor) too. ANOVA statistics was performed to evaluate the relationships between adiponectin, EE, lipid metabolism and insulin resistance in the different groups.
**Results:** There were no significant differences regarding age and BMI between the groups (table 1). Insulin resistance and EE were significantly higher at the diabetic patients. Adiponectin decreases progressively with increasing glucose intolerance (table 2). In the analysis of correlation with adiponectin to different metabolic parameters, significant negative correlations were found to fasting glucose ($r = -0.481$, $p = 0.000$), PGl´120 ($r = -0.391$, $p = 0.003$), HbA1 c ($r = -0.377$, $p = 0.005$), insulin resistance ($r = -0.408$, $p = 0.015$), TG ($r = -0.328$, $p = 0.019$) and a significant positive correlation to HDL-Chol ($r = 0.540$, $p = 0.000$). We found a significant negative correlation between adiponectin and EE ($r = -0.337$, $p = 0.011$).
**Conclusion:** We could prove the well known association between adiponectin and insulin resistance, especially for IGT and DM. To our knowledge we reported to the first time a significant negative correlation between adiponectin and energy expenditure.

Table 1 population characteristic (mean and standard deviation)

| | NGT | IGT | DM | p |
|---|---|---|---|---|
| n | 20 | 20 | 20 | |
| age | 60.1 (7.8) | 58.6 (6.3) | 56.6 (9.2) | n.s. |
| BMI | 26.9 (3.6) | 28 (4.1) | 28.1 (3.6) | n.s. |
| PG 0` | 5.3 (0.41) | 6.0 (0.57) | 7.3 (1.0) | 0.000 |
| PG 120´ | 5.3 (1.3) | 9.1 (0.8) | 11.8 (3.1) | 0.000 |
| HbA1 c | 5.3 (0.3) | 5.4 (0.3) | 5.9 (0.7) | 0.002 |

Table 2 Results (mean and standard deviation)

| | NGT | IGT | DM | p |
|---|---|---|---|---|
| Insulin 0` | 10.6 (5.0) | 11.7 (4.0) | 15.66 (4.4) | 0.024 |
| Insulin 120` | 41.9 (22.1) | 65.7 (43.3) | 98.4 (66) | 0.033 |
| HOMA-R | 2.6 (1.3) | 3.1 (1.1) | 5.1 (1.6) | 0.000 |
| Adiponectin | 13.9 (6.9) | 11.1 (4.8) | 8.6 (6.8) | 0.041 |
| TG | 1.17 (0.5) | 1.45 (0.6) | 1.78 (0.9) | n.s. |
| HDL-Chol | 1.73 (0.47) | 1.53 (0.35) | 1.38 (0.46) | n.s. |
| EE (kcal/24 h) | 1428.9 (195.3) | 1577 (274.7) | 1649 (287.6) | 0.031 |
| EE (kcal/kgLBM) | 28.9 (2.8) | 30.7 (4.6) | 29.0 (3.5) | n.s. |

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# Metabolic syndrome: inflammation

## 619

**Aging-induced impairment of antioxidant enzyme activity in non-obese model of metabolic syndrome.**

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**Background and aims:** Insulin resistance as well aging are associated with an increase risk of cardiovascular diseases (CVD), but mechanisms involved are not completely understood. Growing evidence indicate that oxidative stress plays a central role in the pathogenesis of CVD. In this study the metabolic impact of insulin resistance and aging on antioxidant enzymes activity and reduced glutathione (GSH) level in aorta and myocardium in 3- and 12-months old rats of hereditary hypertriglyceridemic (HHTg) strain and control rats were compared.
**Materials and methods:** The strain of HHTg rats, originating from Wistar strain, exhibits in addition of hypetriglyceridemia other symptoms of insulin resistance syndrome such impaired glucose tolerance and insulin action, hyperinsulinemia and elevated blood pressure. Random-bred normotriglyceridemic (NTg) Wistar rats of the same age were used as controls. Groups of male rats were fed for two weeks a high carbohydrate diet (70 cal % sucrose).
**Results:** Serum triglycerides level was elevated in HHTg rats in comparison with NTg rats at age of 3-mo ($5.01 \pm 0.73$ vs $1.67 \pm 0.13$ mM/l, $p<0.05$). In 12-mo old animals the changes in serum triglycerides level were similar ($5.56 \pm 0.47$ mM/l in HHTg vs $1.27 \pm 0.08$ mM/l in NTg mM/l, $p<0.05$). Antioxidant enzymes activity superoxiddismutase (SOD), catalase (CAT) and seleno-dependent glutathione peroxidase (GSH-Px) in myocardium were reduced in 3-mo-old HHTg rats in comparison to NTg rats at same age and further decreased in 12-mo old HHTg rats (table). The results indicate that SOD and CAT activity in 12-mo old NTg rats was significantly decreased in comparison 3-mo old NTg rats. In contrast, in HHTg rats aging-induced inhibition was found only for GSH-Px activity. Aortic GSH level was markedly lower in 3-mo old HHTg rats and was further reduced by aging in NTg rats as well in HHTg rats.
**Conclusion:** Hypertriglyceridemia is associated with increased oxidative stress in aorta and myocardium, showed as reduction of antioxidant enzymes activity and GSH level. Aging potentiated the negative effect of hypertriglyceridemia on reduced glutathione level, a key component of antioxidant defense. These disorders may contribute to development of vascular diseases associated with metabolic syndrome.

Heart antioxidant enzymes activity and aortic GSH level

| | NTg | | HHTg | |
|---|---|---|---|---|
| Age, months | 3 | 12 | 3 | 12 |
| SOD U/mg protein | $1.03 \pm 0.01$ | $0.83 \pm 0.11^{a}$ | $0.68 \pm 0.06^{c}$ | $0.55 \pm 0.05$ |
| CAT, μM $H_2O_2$/min/mg protein | $812 \pm 36$ | $499 \pm 57^{a}$ | $530 \pm 59^{c}$ | $425 \pm 52$ |
| GSH-Px, μM GSH/min/mg protein | $831 \pm 22$ | $767 \pm 44$ | $738 \pm 36$ | $627 \pm 38^{b,d}$ |
| GSH in aorta, nM/g | $0.77 \pm 0.09$ | $0.46 \pm 0.04^{a}$ | $0.49 \pm 0.04^{c}$ | $0.24 \pm 0.03^{b,d}$ |

[a]- $p>0.05$ NTg at age 3-mo vs 12- mo
[b]- $p>0.05$ HHTg at age 3-mo and 12-mo
[c]- $p>0.05$ 3-mo old NTg vs HHTg
[d]- $p>0.05$ 12-mo old NTg vs HHTg

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## 620

**Fenofibrate improves the atherogenic lipid profile and markers of vascular inflammation independent of changes in insulin sensitivity in hypertriglyceridemic subjects with the metabolic syndrome**

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**Background and aim:** Fibrates have been reported to improve the atherogenic dyslipidemia of insulin resistant hypertriglyceridemic (HTG) subjects and reduce coronary artery disease, but the mechanism(s) remain incompletely understood. Whether amelioration of insulin resistance may be an important mechanism of action of fibrates deserves further evaluation.

**Method:** To this end, we randomized (2:1, double-blind) 25 non-diabetic insulin resistant HTG pts (age: 46 ± 3 yr, BMI: 31 ± 1 kg/m$^2$, FPG: 95 ± 2 mg/dl, FPI: 13 ± 2 μU/ml, TG: 435 ± 54 mg/dl, HDL-C: 34 ± 2 mg/dl) to FENO (200 mg/d; n= 16) or placebo (PBO; n= 9) in a 12-week trial. Following a 4-week run-in period and after treatment, lipid metabolism (apolipoprotein profile) and glucose turnover (80 mU · m$^{-1}$ · min$^{-1}$ euglycemic insulin clamp with $^3$H glucose) were assessed using gold standard methods. We also measured markers of vascular inflammation (CRP, IL-6, VCAM, ICAM).
**Results:** Weight remained stable during the study. There were no changes in glucose/lipid metabolism with PBO. In contrast, FENO led to a less atherogenic lipid profile as it decreased fasting TG by 59% (500 ± 71 to 207 ± 24 mg/dl, p<0.001) and increased HDL-C by 6% (p<0.06). This was associated with an increase in Apo AI and AII and a 45% reduction in Apo CII and Apo CIII (p<0.01). The reduction in TG correlated with the reduction in Apo CIII (r = 0.50, p<0.01) and correlated inversely with an increase in HDL-C (r = 0.59, p<0.01). FENO decreased fasting FFA by 34% (700 ± 48 to 464 ± 35 mg/dl, p<0.002) and enhanced FFA suppression by insulin (222 ± 39 vs. 117 ± 12 mM, p<0.02). FENO was associated with a reduction in markers of vascular inflammation: CRP (–34%, p<0.03), IL-6 (–27%, p<0.04), ICAM (–5%, p<0.05) but VCAM did not change significantly. Fasting plasma glucose, insulin and endogenous glucose production (EGP) were not reduced by FENO, while whole body insulin-stimulated glucose disposal (Rd) did not increase significantly (3.7 ± 0.3 vs. 4.2 ± 0.5 mU · m$^{-1}$ · min$^{-1}$, p = 0.11). The modest change in Rd did not correlate with the reduction in plasma TG, CRP, IL-6, VCAM or ICAM concentration.
**Conclusions:** In HTG insulin resistant patients, FENO improves the apolipoprotein profile towards a less atherogenic pattern and decreases vascular inflammation without a significant change in glucose metabolism. Therefore, independent of changes in insulin sensitivity, FENO induces lipid/vascular effects that are likely to reduce the high cardiovascular risk of these patients.

## 621

**Minor contribution of blood glucose control to dyslipidaemia, oxidative stress and insulin resistance in Type 2 diabetic women with metabolic syndrome**

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**Background and aims:** Insulin resistance (IR) is associated with both type 2 diabetes mellitus (T2D) and cardiovascular disease. However, the relative effect of hyperglycaemia on cardiovascular risk factors connected with IR (dyslipidaemia, oxidative stress) has still remained unknown. In this study we aimed to determine whether improved glycaemic control significantly impacted on atherogenic lipid profile and oxidative stress parameters in insulin resistant T2D women (T2DW).
**Materials and methods:** A total of 159 T2DW (age 54.0 ± 0.5 years, diabetes duration 7.5 ± 0.5 years, BMI 30.6 ± 0.4 kg/m$^2$, WHR 0.87 ± 0.004) with dyslipidaemia (serum triglycerides 4.05 ± 0.06 mmol/l, total cholesterol 7.20 ± 0.09 mmol/l, LDL-cholesterol 4.57 ± 0.09 mmol/l, HDL-cholesterol 0.80 ± 0.01 mmol/l), who were not taking lipid-lowering therapy, were enrolled in the study and divided in three groups with regard to their glycaemic state: 62, 41, and 56 T2DW under good (HbA1 c < 6.5%), moderate (HbA1 c ≤ 7.5%) and poor (HbA1 c > 7.5%) glycaemic control, respectively. Lipid peroxidation products (total performed lipid peroxides-ROOHs, conjugated dienes – CD, thiobarbituric acid reactive substances – TBARS) and antioxidative activities (serum paraoxonase – PON, catalase, SH-glutathione; α-tocopherol content in VLDL+LDL or apoB and HDL) were measured by spectrophotometry. Indices of IR were determined using the HOMA-algorithm and Quantitative Insulin Sensitivity Check Index (QUICKI). 21 healthy, age-matched women with BMI 26.8 ± 0.8 kg/m$^2$ and WHR 0.78 ± 0.01 served as controls.
**Results:** Compared to controls T2DW had strong significantly (p<0.001) higher values for serum triglyceride (by 150%), total cholesterol (by 30.9%), CD (by 373.3%), TBARS (by 244.1%), HDL-ROOHs (by 174.3%) and apoB-ROOHs (by 116.8%) as well as lower serum HDL-cholesterol levels (by 33.3%), α-tocopherol content in HDL and apoB (by 64.7% and 44.1%, respectively) and serum PON activity (by 37.9%). IR was verified in all T2DW (HOMA-IR index 8.65 ± 0.55 vs 3.30 ± 0.31, QUICKI 0.460 ± 0.005 vs 0.561 ± 0.021 in controls, p<0.001). Moreover, progressive decrease in IR was observed when moving from T2DW with poor to good glycaemic control (HOMA-IR index 11.15 ± 1.01 vs 6.22 ± 0.67, QUICKI 0.454 ± 0.013 vs 0.516 ± 0.014, respectively, p<0.001). In comparison with poorly controlled T2DW well controlled ones had also a significant albeit small decrease in serum triglycerides (by 7.1%), total cholesterol (by 9.2%) and LDL-cholesterol (by 10.2%) levels and attenuation of oxidative stress parameters: serum CD, TBARS, HDL-ROOHs and apoB-ROOHs were decreased by 14.1%, 14.3%, 14.3% and 11.23% respectively, PON activity and α-tocopherol content in both HDL and apoB were increased by 12.7%, 24.3% and 27.7%, respectively. But any above mentioned parameters verified in T2DW under good glycaemic control did not reached the controls group levels (p<0.001).
**Conclusion:** In Type 2 diabetic women against the background of metabolic syndrome good glycaemic control is insufficient to produce important change of the abnormal lipid profile, oxidative stress and to rehabilitate insulin sensitivity. We suggest that glucotoxity has the minor impact on development of insulin resistance and last one could significantly contribute to the genesis of dyslipidaemia and oxidative stress in Type 2 diabetic women.

## 622

**Modulation of insulin-like growth factor-I and their binding proteins by insulin resistance-associated inflammatory activity**

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**Background and aims:** Insulin resistant subjects have been shown to exhibit both a low-grade chronic inflammatory activity and changes in the insulin-like growth factor (IGF) system. Because the IGF system is an important modulator of insulin sensitivity, we wished to study the cross-sectional associations between pro- and anti-inflammatory markers, the IGF-IGFBP system and insulin sensitivity in human subjects.
**Materials and methods:** Healthy [n=60; age 53.4 ± 10.7; body mass index (BMI) 25.2 ± 1.7)], obese (n=50; age 53.6 ± 9.6; BMI 30.8 ± 2.4) and type-2 diabetic (n=42; age 57.7 ± 9.0; BMI 29.3 ± 3.4) Caucasian men were studied as part of a population-based study dealing with insulin sensitivity in Northern Spain. Serum interleukin-6 (IL-6) was measured by ultrasensitive chemiluminescent immunometric assay; soluble tumor necrosis factor-α receptor 1 (sTNFR1) and 2 (sTNFR2) by EASIA; adiponectin by RIA; and total IGF-I, free IGF-I, IGFBP-1 and IGFBP-3 by IRMA. Insulin sensitivity was assessed by intravenous glucose tolerance test with minimal model analysis in non-diabetic subjects.
**Results:** By one-way ANOVA, the three groups differed in serum IL-6 (p=0.014), adiponectin (p=0.004), total and free IGF-I (p<0.0001), IGFBP-3 (p=0.001) and IGFBP-1 concentrations (p=0.002). These differences persisted after adjustment for age and body mass index (BMI) for all analytes except for IL-6. Significant correlations were observed between insulin sensitivity and free IGF-I (r=0.54, p=0.002) and IGFBP-1 (r=0.37, p=0.04). In addition, IL-6 correlated negatively with free IGF-I (r= –0.38, p=0.02) and sTNFR1 correlated positively with IGFBP-1 (r=0.41, p=0.01).
In multivariate analysis both IL-6 and sTNFR1 were independent predictors of free IGF-I, explaining 18% and 10% of its variance, respectively (excluded variables: age, BMI and adiponectin). In another model, BMI, age and sTNFR1 were independent predictors of IGFBP-1, explaining 31%, 16% and 8% of its variance, respectively (excluded variables: IL-6 and adiponectin). The predictive value of the proinflammatory parameters was lost when insulin sensitivity was substituted for adiponectin in these models. Moreover, insulin sensitivity stood as the only predictive variable for free IGF-I, explaining 24% of its variance.
**Conclusion:** Proinflammatory cytokines, by causing insulin resistance, may modulate serum concentrations of free IGF-I in men.
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## 623

**Interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α) and soluble TNF receptor 1 (sTNFR1) in healthy twins – aetiology and relation to in vivo insulin action.**

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**Background and aims:** Cytokines may play distinct roles in the regulation of glucose homeostasis including insulin action in man. The aim was to examine the impact of genetic versus pre- and post natal environmental factors on the plasma levels of the cytokines IL-6, TNF-α and sTNFR1, and to investigate the association of these cytokine levels to insulin sensitivity in twins.
**Materials and methods:** IL-6, TNF-α and sTNFR1 levels were measured in fasting plasma samples from 196 monozygotic (MZ) and dizygotic (DZ) twins by ELIZA (R&D systems). Insulin sensitivity was measured using the euglycaemic hyperinsulinaemic clamp technique. Heritability estimates and intra pair correlations were calculated, and multiple regression analyses were performed, to examine the association between cytokine levels and antropometric and metabolic measurements.
**Results:** No significant impact of genetics was found for any of the measured cytokines. There was a significant intra-pair correlation between birth weight (Bw) and IL-6 levels among both monozygotic and dizygotic twins ($MZ_{total}$: r=−0.45, p<0.0001; $DZ_{total}$: r=−0.24, p=0.03) and between sTNFR1 levels and BW among MZ twins only ($MZ_{total}$: r=−0.28, p=0.004). TNF-α was not associated with Bw. An intra-pair correaltion between IL-6 and Rd was found in MZ twins (r=−0.22, p=0.02). The regression analysis demonstrated a significant effect of Bw and age on IL-6 levels with no detectable impact of sex, zygosity, BMI, Rd or fat %. The plasma TNF-α and sTNFR1 levels were not significantly associated with Bw, although an effect of zygosity, age and sex on sTNFR1 was seen.
While simpel correlation analysis documented a negative correlation between IL-6 level and insulin action in vivo (Rd) and between sTNFR1 and Rd, these correlations disappeared after correction for other covariates known to influence insulin action. TNF-α were not signifantly associated with Rd.
**Conclusion:** Cytokine levels in plasma are primarily regulated by non-genetic factors, including a major influence of the intrauterine environment on IL-6, and to some extent on sTNFR1 levels, but not on TNF-α levels. Our data does not support any clinical important impact of the cytokine level per se for insulin resistance in healthy non-diabetic twins.

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**Association of the metabolic syndrome and profile of inflammatory cytokines in Type 2 diabetes**
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Evidence suggests that atherosclerosis and T2D are associated with a state of chronic inflammation, reflected by a rise of acute phase response markers and inflammatory cytokines like TNF-α and IL-6. TNF-α induces insulin resistance and is associated with hyperinsulinaemia. Our cross-sectional study of subjects with T2D investigated whether features of the metabolic syndromes influence the levels of inflammatory marker and whether insulin-sensitizing drugs or statins have any effect on those levels.
**Methods:** 53 patients (age range: 45–79, BMI-range: 21.3–60) with T2D were recruited from the outpatients clinic. CRP, TNF-α, interleukin-6 (IL-6), interleukin-8 (IL-8) beside a full lipid profile and HbA1 c were measured in their sera. Urine was collected overnight for measurement of albumin excretion rate. Anthropometic parameters (weight, height, waist-circumference) were measured and their blood pressure recorded. Drug history, smoking status, year of diagnosis of T2D, presence or absence of hypertension and coronary heart disease (CHD) were documented. 29 healthy volunteers (age-range: 20–52, BMI-range 18.6–41.1) served as controls.
**Results:** 82% of patients had the metabolic syndrome according to modified WHO criteria (≥ two of: BMI >30 kg/m²; BP>140/90 or anti-hypertensive treatment (AHT); HDL-cholesterol <0.9 in men, <1.0 mmol/L in women; triglycerides ≥ 1.7 mmol/L, presence of microalbuminuria). 48% fulfilled the criteria for the metabolic syndrome according to the National Cholesterol Education Program (NCEP) (≥ three of: waist-cf: > 88 cm in women, >102 in men; BP≥ 130/85 or AHT; HDL < 1.04 in men, < 1.29 mmol/L in women; triglycerides ≥ 1.7 mmol/L). CRP and IL-6 were significantly higher in the T2D group even after allowing for age, BMI and waist-cf. (mean CRP 3.16 mg/L vs 1.8 mg/L; mean IL-6 3.99 pg/ml vs 1.14 pg/ml; p < 0.05). CRP, IL-6 and TNF-alpha in the T2D group were all positively correlated with serum cholesterol and cholesterol/HDL ratio respectively (r=0.33, r=0.51, r=0.31, p < 0.05), while only CRP was positively correlated with HbA1 c (r=0.29, p = 0.04). In a multiple regression analysis with IL-6 as the dependent variable and cholesterol, triglycerides, HDL/cholesterol ratio and TNF-α as the independent variables, only cholesterol and TNF-α were independent predictors of the level of IL-6 in T2D (adj. $R^2$ = 0.315, P< 0.0001). Subjects with the metabolic syndrome (WHO-criteria) had higher levels of IL-6 and CRP when compared to those without the syndrome (IL-6: mean 4.66 vs 0.78; CRP: mean 3.53 vs 1.37; p<0.03). However, no difference in cytokine levels was observed when the NECP criteria were applied. No significant treatment effect of insulin-sensitizing drugs or statins on levels of cytokines was observed in our study.
**Conclusion:** Our study has demonstrated increased levels of IL-6 and CRP in T2D, but in contrast to other studies, TNF-α and IL-8 were not raised. Inflammatory markers were positively associated with an adverse lipid profile and poor glycaemic control was reflected by a raised CRP. Our study suggests that the WHO criteria for the metabolic syndrome are a better discriminator of an adverse inflammatory cytokine profile in T2D when compared to the NECP criteria. Our study supports previous evidence that dyslipidaemia and hyperglycaemia in T2D is associated with a chronic inflammatory response and warrants aggressive management.

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**Postprandial adiponectin levels after an oral lipid tolerance test versus oral glucose tolerance test and association with parameters of the metabolic syndrome**
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**Background and aims:** Type 2 diabetes and obesity are associated with an alteration of insulin response on target tissues. The adipose-specific hormone adiponectin was described as the possible link between obesity and insulin resistance via an insulin sensitizing action. In human studies low adiponectin levels were associated with high insulin and triglyceride levels, insulin resistance and other features of the metabolic syndrome. In vivo, adiponectin 16 kDa fragment has short-term effects on glucose and fatty acid levels. There is little information on the acute postprandial regulation of adiponectin. Because of strong associations between postprandial triglycerides with an early insulin resistance syndrome we examined the association of metabolic features with postprandial adiponectin levels.
**Materials and methods:** 149 male patients with normal fasting plasma glucose levels and a mean age of 55 yrs were investigated. Blood was collected after an oral glucose tolerance test at 0,5, 1, 2, 3 and 4 hours and, on another day, after an oral high-fat drink (51,6 kcal% fat, 29,6 kcal% carbohydrates, 11,9 kcal% protein), at 0,5, 1, 2, 3, 4, 5, 6, 7, 8 and 9 hours postprandially. Adiponectin was measured using ELISA (R&D-systems).
**Results:** Adiponectin levels decreased postprandially after both test meals. Significance level (Bonferroni-Holm) was attained 5 and 6 hours postprandially after the lipid load. After the glucose load a significant decrease of adiponectin was seen earlier (30 min. to 120 min.) postprandially. In both tests, adiponectin showed a negative correlation with postprandial insulin levels and HOMA and positive correlation (R=0.27, p=0.007) with HDL-cholesterol. After the lipid load the association of adiponectin with postprandial triglycerides and insulin and HOMA was stronger than with fasting values: R=0.22 (p=0.027) with postprandial insulin (AUC); R = 0.10 (n.s.) with fasting insulin; R = 0.28 (p = 0.03) with postprandial HOMA (AUC), R = 0.17 (u.s.) with fasting HOMA; R = 0.26 (p = 0.007) with postprandial TG (AUC) and R = 0.20 (p = 0.04) with fasting TG.
**Conclusion:** The decrease of adiponectin concentrations after glucose and fat ingestion might play a role in the pathogenesis of the metabolic syndrome.
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**Adiponectin concentration and TNF-α system activity in lean nondiabetic offspring of Type 2 diabetic subjects**
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**Background and aims:** There is a growing evidence that adiponectin function is related to the pathogenesis of insulin resistance. Insulin resistance, however, may precede the development of obesity in subjects with strong family history of type 2 diabetes. The aim of the present study was to look for adiponectin role in pathogenesis of insulin resistance in offspring of type 2 diabetic patients and its relation to TNF-α system activity.
**Materials and methods:** The study was carried out in 18 lean offspring of type 2 diabetic subjects and 16 controls matched for age, sex and BMI. The OGTT with glucose and insulin estimations and hyperinsulinemic, euglycemic clamp were performed in all patients. The plasma

concentration of adiponectin, TNF-α, sTNFR1, sTNFR2, HbA1 c, total cholesterol, HDL-cholesterol, LDL-cholesterol and TG concentrations were also estimated.
**Results:** Insulin sensitivity index ($M_{ffm}$), calculated from the clamp studies, in offspring of type 2 diabetic subjects was markedly decreased (p=0.008) in comparison to the control group. Plasma adiponectin concentration was slightly higher in the control group, this difference did not reach the level of significance (p=0.11). TNF-α and sTNFR1 concentration was similar in both groups. The concentration of sTNFR2 was markedly increased in group of type 2 diabetic offspring (p=0.000097). Adiponectin concentration were inversely related to WHR (r=–0.367, p=0.034), glucose concentration in 30 minutes of OGTT (r =–0.44, p=0.013), sTNFR2 (r=–0.46, p=0.012) and to sTNR1 of borderline significance (r =–0.32, p=0.086). Positive correlation was observed with HDL-cholesterol concentration (r=0.37, p=0.03). Multiple regression analysis revealed that HDL-cholesterol, sTNFR2 and fasting insulin were independent predictors of adiponectin concentration and were responsible for 44% of its variability.
**Conclusion:** The obtained results suggest that adiponectin could play a role in the pathogenesis of insulin resistance in lean offspring of type 2 diabetic subjects, but its concentration is dependent on TNF-α system activity.
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## 627

**Diabetes is the main factor accounting for the high ferritin levels detected in patients with chronic hepatitis due to C virus infection**
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**Background and aim:** A high prevalence of diabetes has been reported in patients with hepatitis C virus (HCV) infection. Both diabetes and HCV infection are associated with high serum ferritin levels. Although HCV infection could be the main factor responsible for the high ferritin levels it is also possible that diabetes rather than HCV infection might be a major contributor to the high ferritin levels observed in HCV infected patients.The aim of the present study was to investigate the contribution of diabetes to the high ferritin levels observed in HCV infected patients with chronic hepatitis.
**Material and Methods:** 619 non-cirrhotic individuals were prospectively recruited. According to the HCV antibody status and the presence of diabetes, the subjects were divided in 4 groups: group A (anti-HCV positive diabetic patients, n=46), group B (anti-HCV negative diabetic patients, n=236), group C (anti-HCV positive non-diabetic patients, n=173), and group D (anti-HCV negative non-diabetic control subjects, n=164). Serum ferritin levels, and other iron metabolic parameters were determined. Multiple regression analyses were performed to explore the variables independently related to ferritin levels.
**Results:** Serum ferritin levels in group A were significantly higher than in the other groups (A>B, p<0.01; A>C, p<0.001; A>D, p<0.001). Group B showed higher ferritin levels than group D (p=0.001). However, group C has similar ferritin values to group D. In multivariate analyses diabetes but not HCV infection was independently related to serum ferritin concentrations.
**Conclusions:** Diabetes rather than HCV infection itself is the main factor accounting for the increased ferritin levels detected in HCV infected patients. Therefore, the presence of diabetes should be taken into account when evaluating iron metabolism in HCV infected patients.
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## 628

**Ferritin and insulin resistance**
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**Background and aims:** Previously we demonstrated that patients with type 2 diabetes have higher plasma ferritin levels than type 1 diabetics. Indeed, in many epidemiological studies plasma ferritin concentration significantly correlated with certain markers of insulin resistance syndrome: body mass index (BMI), hyperglycemia, hypertension, hypertriglyceridemia and low HDL-cholesterol. However, it is still not known whether ferritin plays a causal role in the pathogenesis of the metabolic syndrome or if it is just a marker of heme iron consumption in high caloric diet in general
Aim of the study: Comparison of two types of reduction diet which differ in the amount of heme iron and their impact on changes in body iron stores and insulin sensitivity.
**Materials and methods:** Obese subjects (BMI>25 kg/m$^2$) aged 25–65 years were included in the study and randomized according to the type of weight reduction diet into 2 groups: Diet A (with relative energy substrate distribution P:F:C = 10:45:45 percent) and diet B (with P:F:C=15:30:55 percent). Diet A contained 5% less protein (as meat) than diet B. The dietary energy load was assessed using a formula: BEE*1.5–600 (kcal). BEE (basic energy expenditure) was estimated in every subject under standard conditions using indirect calorimetry. Group on type A diet: 14 subjects (4 men, 10 women), group B 25 subjects (10 men and 15 women); sex distribution between the groups did not differ statistically ($\chi^2$=0.51, p=0.4754). In all respondents, plasma ferritin and transferrin concentrations were measured. Insulin resistance was estimated using fasting glycemia, fasting C-peptide, ratio C-peptide/glycemia, triglycerides, HDL-cholersterol.
**Results:** After 3 months dietary intervention, only in the group A there was a significant decrease of plasma ferritin. The mean difference before and after the intervention was 34.27 ± 53.21 ng/ml, p>0.05 in the group A and –0.29 ± 104.559 ng/ml in the group B. However, both A and B dietary regimens had a similar effect on weight reduction (2.27 ± 1.114 kg/m$^2$, p<0.01; resp. 2.21 ± 1.440 kg/m$^2$, p<0.01), decrease in fasting glucose (1.22 ± 1.993 mmol/l, p<0.05; resp. 1.04 ± 1.674 mmol/l, p<0.01), decrease in C-peptide (356.57 ± 218.676 pmol/l, p<0,01; resp. 468.56 ± 430.663 pmol/l, p<0.01), decrease in C-peptide/glycemia ratio (54.00 ± 55.334, p<0.01; resp. 60.49 ± 89.019, p<0.01). In the whole cohort. there was no correlation between plasma ferritin and fasting C-peptide/glycemia ratio.
**Conclusion:** Our results demonstrated that the decrease of ferritinemia in A group is caused by a lower content of meat and thus heme iron in this type of diet and that ferritin does not correlate with insulin sensitivity. We thus suppose that elevated ferritin levels, traditionally described in syndrome of insulin resistance and type 2 diabetes, are in no causal relationship with metabolic syndrome and represent either epiphenomenon than consequence of this disorder.

# PS 48

# Metabolic syndrome: experiments in animals

## 629

**Maternal social stress increases insulin resistance and dislipidaemia in male offspring of gestational diabetes mothers**

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**Background and aims:** Insulin resistance is a very important risk factor for many metabolic disorders including type 2 diabetes mellitus, obesity and cardiovascular diseases. Impaired insulin sensitivity was found in adult offspring of rats with streptozotocin (STZ)-induced gestational diabetes (GD). the study was conducted to evaluate the impact of maternal stress against the background of GD on insulin resistance and dislipidaemia in male offspring ($F_1$) at the sex-maturity period.
**Materials and methods:** For MSS creation rats were transferred daily from one association to another within $2^{nd}$-$8^{th}$ day of pregnancy, GD was rendered by a single streptozotocin injection (45 mg/kg b. w., i.p.) on the second day of pregnancy. The maternal cohort consisted of 25 pregnant rats exposed to MSS, GD, MSS+GD and controls (C). An i.p. glucose tolerance test (GTT, 3g glucose/kg b. w.; 0, 30, 60, 120 min) was performed after an overnight fast in male $F_1$ (n=32) of analogous groups at 90 days of age. Fasting blood samples were used for glycemia, plasma insulin (IRI), non-esterified fatty acids (NEFA), triglyceride (TG) and total cholesterol (TCh) levels determination. insulin resistance (IR) state was estimated using Homeostasis Model Assessment (HOMA), insulin sensitivity was evaluated using Quantitative Insulin Sensitivity Check Index (QUICKI).
**Results:** Decrease in glucose tolerance observed in GD $F_1$ was more pronounced in MSS+GD $F_1$ (AUC/2 h over GTT: $1022.7 \pm 19.3$ vs $1112.0 \pm 32.8$ mmol/l/min, respectively ($p<0.05$), in comparison with $640.7 \pm 8.0$ mmol/l/min in C $F_1$ ($p<0.001$)). Plasma IRI and HOMA-IR in the MSS+GD $F_1$ were increased by 22% and 34% respectively ($p<0.01$), compared to the GD $F_1$ and by 140% and 292% respectively, compared to the C $F_1$ ($p<0.001$). MSS reduced QUICKI in GD $F_1$ ($0.215 \pm 0.003$ vs $0.225 \pm 0.004$, $p<0.05$; C $F_1$: $0.271 \pm 0.004$, $p<0.001$). TCh and NEFA levels were significantly enhanced both in GD $F_1$ and MSS+GD $F_1$ compared to C $F_1$ (TCh: $2.68 \pm 0.18$ and $2.63 \pm 0.15$ mmol/l vs $1.78 \pm 0.12$ mmol/l in C $F_1$, respectively, $p<0.001$; NEFA: $1.26 \pm 0.06$ and $1.20 \pm 0.07$ mmol/l vs $0.49 \pm 0.11$ mmol/l in C $F_1$, respectively, $p<0.001$). MSS increased TG in GD $F_1$ ($0.225 \pm 0.004$ vs $0.215 \pm 0.003$ mmol/l, $p<0.05$; C $F_1$: $0.271 \pm 0.004$ mmol/l, $p<0.001$).
**Conclusion:** These results demonstrate that maternal social stress against the background of gestational diabetes strengthened insulin resistance (according to HOMA-IR and QUICKI) and dislipidaemia in first generation rats male offspring at the sex-maturity period.

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**Low protein diet during gestation and lactation in rats predisposes to visceral adiposity and insulin resistance in peripherial tissues in early adulthood with a dramatic effect in males**

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**Background and aims:** Dietary restriction during pregnancy retards physical growth resulting in small body size. Rats treated with a restricted low protein diet (LPD) 8% during pregnancy and lactation have a reduced β cell mass at birth and a reduced insulin secretion later in life, lower birth weight and weight gain in adult life, being ($p<0.01$) significative in both males and females.
All animals were switched to a normal control (C) 20% diet after weaning. The aim of this study was to investigate the role of nutrition in programming fetal and neonatal rats and the impact of this, on glucose homeostasis later in life and to determine the timing of changes in the response to insulin in peripheral tissues.
**Materials and methods:** The response to an intraperitoneal (i.p.) glucose tolerance test (IGTT) 80 and 130 days of age was studied. Both treatments and sexes were analized. Animals were anaesthesized with Ketamine/Xylazine at 130 days and treated with an insulin challenge by injecting insulin into the portal vein (2U/kg) and sacrificing the rats at 1 minute. Muscle and adipose tissues were homogenized; proteins extracted and western blots were assessed for the phosphorylation of PKB. By DNA microarray genes were assayed in adipose tissue at 130 days to study genetic changes in the (LPD) male rats.
**Results:** Glucose tolerance test (IGTT) at 80 days was within normal values. At 130 days, this response changed in LPD group with a 14% AUC (area under the curve) in males and 42% AUC in females, while basal insulin levels were 2 fold higher in males of the LPD group. By HOMA IR* and HOMA β*-cell the LPD males were significantly higher $p<0.04$ (Mann-Whitney non-parametric test). Fat/Body weight in the LPD group was higher in LPD group specially in the LPD males. By red oil O staining livers of female and male LPD rats showed less fat infiltration compared to the normal controls. Western blotting showed a reduced insulin-induced phosphorylation of PKB in fat (bigger effect in males than females) in the LPD group and also a reduced insulin-induced PKB phosphorylation in muscle and livers only from males of the LPD. Results of the DNA microarray are shown in table 1 and the increased expression was validated by quantitative real time RT-PCR.
**Conclusion:**
The results suggest that low protein diets during gestation and lactation can predispose rats in young adulthood to visceral adiposity, reduced PKB phosphorylation predominantly in fat tissues and gene expression that combined can induce insulin resistance with a more dramatic effect in males.

Genes that are differentially expressed in visceral adipose tissue of male LPD rats at 130 days.

| Gene | Fold change by microarray | Fold change by real time RT-PCR |
|---|---|---|
| LPL | +1.1 | +1.2 |
| C/EBP-alpha | +1.3 | +1.2 |
| C/EBP-beta | +1.6 | +2.7 |
| C/EBP-delta | +1.9 | +3.5 |
| Leptin | +1.9 | +3.3 |
| GLUT-4 | +1.7 | +4.9 |
| IRS-1 | -1.4 | -1.7 |
| 11 beta-HSD1 | +1.3 | +1.4 |

* Insulin resistance HOMA IR: Fasting insulin (μU/ml) X fasting glucose (mmol/l) / 22.5
* β- cell function HOMA β-cell: 20 X fasting insulin (μU/ml) / fasting glucose (mmol/l) -3.5

*Supported by: Canadian Diabetes Association*

## 631

**The castrated male Sprague-Dawley rat as a model of the metabolic syndrome and Type 2 diabetes**

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**Background and aims:** Sex hormones influence body composition and body fat distribution in humans, and both are thought to influence the metabolic consequences of obesity. The aim of this study was to describe the immediate and subchronic effects of testosterone deficiency on cumulated food intake (FI), body weight (BW), energy expenditure (EE), activity level, body fat distribution and selected plasma parameters in male rats to investigate the suitability of the rat as a model for the human situation in this respect.
**Materials and methods:** Male Sprague-Dawley rats were either castrated or sham-operated at 16 weeks of age (n=11). FI and BW were recorded twice weekly, blood samples for measurement of plasma parameters and CT-scanning to determine total abdominal fat area (TFA), visceral fat area (VA), subcutaneous fat area (SA) and SA/VA was done 1–2 weeks and 9–10 weeks after castration/sham-operation. EE, activity and glucose tolerance (OGTT) were measured 8–10 weeks after castration/sham-operation.
**Results:** FI and BW did not differ at any time point. Glucose was higher and FFA and glycerol lower in the castrated rats 2 weeks after castration. For glucose and FFA this relative difference between the groups was maintained throughout the study. HbA1 c was increased in the castrated rats in the end of study supporting the glucose data. CT scanning showed an increase in VA and SA in both groups from week 2 to week 10, but the castrated rats gained more subcutaneous fat than the sham-operated rats, which resulted in a lower SA/VA in this group in the end of the study. No differences were found between the groups for insulin, TG and cholesterol at any time point. No differences were found in OGTT, EE and activity.

**Conclusion:** Castration resulted in lower FFA and glycerol levels, increased fasting blood glucose and increased HBA1 c. These changes seemed to be mediated directly by the lack of testosterone. Testosterone deficiency did not influence VA, whereas in humans visceral fat area measured by CT-scanning is negatively correlated with testosterone concentration. These findings suggest that in the rat absence of testosterone cause accumulation of subcutaneous fat rather than visceral fat and therefore the rat might not be a suitable model for studies on the influence of testosterone on body fat distribution and the following metabolic consequences.
*Supported by: Novo Nordisk A/S*

## 632

### Impaired hepatic nitric oxide pathway contributes for the insulin resistance in obese Zucker rats

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**Background and aims:** Insulin sensitivity is partially dependent on hepatic nitric oxide (HNO). In physiological conditions, N-monomethyl-L-arginine (L-NMMA), a nitric oxide synthase (NOS) competitive inhibitor and 3-morpholinosidnonimine (SIN-1), a nitric oxide (NO) donor, are known to induce and reverse, respectively, insulin resistance, when given intraportally (ipv), but not intravenously. Insulin resistance has been associated with obesity. Since NO action is impaired in animal models of obesity, such as the obese Zucker rat (OZR), we tested the hypothesis that HNO pathway is compromised in OZR, leading to insulin resistance.
**Materials and methods:** Animals were 9 weeks old males. We used lean Zucker rats (LZR) as OZR control. Insulin sensitivity was evaluated through a transient hyperinsulinemic euglycemic clamp, and glucose disposal was calculated as mg glucose/kg body weight. In the basal state, a control clamp was performed, followed by a clamp after administration of L-NMMA (0.73 mg/kg, ipv). SIN-1 (5 mg/kg, ipv) was then administered, followed by a third clamp. Insulin sensitivity dependent on the HNO pathway was assessed by subtracting the response after NOS inhibition (L-NMMA administration) from the basal response and is represented as the resultant insulin sensitivity inhibition.
**Results:** Total insulin sensitivity (basal state clamp) was lower in OZR (79.3 ± 1.6 mg glucose/kg bw; n=6) than in LZR (294.1 ± 21.8 mg glucose/kg bw; n=6; P<0.001). Insulin sensitivity in OZR was 70–75% lower than in LZR (P<0.001). After L-NMMA ipv administration, insulin sensitivity was decreased in the same proportion in both groups (OZR, 48.3 ± 6.6% inhibition; LZR, 45.3 ± 3.5% inhibition). In OZR, SIN-1 did not reverse the L-NMMA-induced insulin resistance, whereas in LZR, SIN-1 induced significant recovery of the insulin sensitivity (89.8 ± 34.9% of the basal state).
**Conclusion:** Our results suggest that functional HNO pathway is essential to maximal insulin sensitivity and that HNO pathway is compromised in obesity, resulting in HNO-dependent insulin resistance. Furthermore, the defect in the HNO pathway seems to be at a downstream point from HNO synthesis.
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## 633

### Intraportal but not intravenous administration of glutathione monoethyl ester improves insulin sensitivity in a dose dependent manner in Wistar rats

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**Background and aims:** The liver modulates insulin sensitivity through the release of the Hepatic Insulin Sensitizing Substance (HISS) that accounts for 55% of peripheral insulin action. Hepatic nitric oxide (NO) and hepatic glutathione (GSH) are both required for normal HISS secretion. In the fasted state, hepatic GSH is decreased and HISS action is impaired, hindering optimal peripheral insulin action.We tested the hypothesis that HISS-dependent insulin sensitivity is improved in a dose dependent manner by intraportal (ipv), but not intravenous (iv), administration of glutathione monoethyl ester (GSH-E), followed by administration of the NO donor, 3-morpholinosidnonimine (SIN-1) to the liver.
**Materials and methods:** Male Wistar rats (8–9 weeks) were fasted for 24 h and anesthetized with pentobarbital (65 mg/kg). GSH-E was administered at the following doses: 0.1, 0.25, 0.5, 1 and 2 mmol/kg bw, both intraportally and intravenously. SIN-1 was administered at a dose of 10 mg/kg, ipv 4. A modified euglycemic clamp was used to quantitate insulin sensitivity in the fasted state and after drug administration (50 mU/kg insulin).
**Results:** In a first group of animals, GSH-E was administered ipv followed by administration of SIN-1 ipv. There was a significant increase in insulin sensitivity, as compared to the fasted state, in a GSH-E dose dependent manner: from 82.4 ± 6.6 to 101.1 ± 13.4 mg glucose/kg bw for a GSH-E dose of 0.1 mmol/kg; from 89.1 ± 18.5 to 146.8 ± 17.2 mg glucose/kg bw for a dose of 0.25 mmol/kg; from 95.2 ± 16.4 to 158.8 ± 19.2 mg glucose/kg bw for a dose of 0.5 mmol/kg, from 83.1 ± 7.5 to 187.3 ± 13.0 mg glucose/kg bw for a dose of 1 mmol/kg and from 76.4 ± 15.6 to 179.9 ± 26.0 mg glucose/kg bw for a dose of 2 mmol/kg, n=23, p=0.02 (ANOVA). In a second group of animals, GSH-E was administered iv followed by SIN-1 ipv. No changes in insulin sensitivity were observed even at the highest dose of GSH-E (2 mmol/kg): from 74.9 ± 3.0 to 75.6 ± 12.8 mg glucose/kg bw for a GSH-E dose of 0.1 mmol/kg; from 86.8 ± 14.9 to 105.7 ± 29.1 mg glucose/kg bw for a dose of 0.25 mmol/kg; from 94.9 ± 9.3 to 99.2 ± 11.8 mg glucose/kg bw for a dose of 0.5 mmol/kg, from 93.8 ± 3.3 to 105.6 ± 9.0 mg glucose/kg bw for a dose of 1 mmol/kg and from 105.4 ± 6.6 to 124.8 ± 15.1 mg glucose/kg bw for a dose of 2 mmol/kg, n=12.
**Conclusion:** Our results are in agreement with the proposed hypothesis that concurrent administration of GSH and NO to the liver is able to enhance peripheral insulin sensitivity on a GSH-E dose dependent manner, on account of HISS action being restored.
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## 634

### The effect of lipid-induced insulin resistance on plasma ghrelin levels in awake rats

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**Background and aims:** Ghrelin is a recently discovered peptide hormone secreted mainly from the stomach. It has a very potent growth hormone (GH)-releasing effect both in animal models and humans. Based on these recent studies, it appears that ghrelin might have a role in glucose and insulin metabolism. Therefore, changes in the activity or concentration of the hormone might constitute a risk factor for impaired glycemic control. However, the results concerning the role of ghrelin in glucose and insulin metabolism are controversial.
Using a lipid infusion in combination with hyperinsulinemic -euglycemic clamps in awake rats to investigate the possible involvement of lipid-induced insulin resistance in rats, we compared plasma ghrelin concentrations before and after clamp established in rats.
**Materials and methods:** A hyperinsulinaemic-euglycaemic clamp was established in awake chronically catheterized rats. Two groups of rats were studied either with a 4-h intraarterial infusion of lipid/heparin (lipid group) or saline (control group). Insulin-mediated peripheral and hepatic glucose metabolism was assessed by insulin clamp combined with [3-$^3$H]-glucose infusion, and plasma ghrelin concentrations were examined before and after clamp. All studies were performed in accordance with the guidelines for the use and care of lab animal of the Chongqing Medical University Animal Care and Use Committee.
**Results:** During hyperinsulinaemic-euglycaemic clamp, there were a significant increase in plasma free fatty acid (FFA, from 741.9 ± 50.6 to 2346.4 ± 238.5 μmol/L, P<0.01) in lipid-infused group. The glucose infusion rates (GIR) in the lipid infusion rats, compared to control rats, were significantly reduced (200 ~ 240 min average: lipid infusion; 12.6 ± 1.5 vs. control; 34.0 ± 1.6 mg/kg.min, P< 0.01) declining to ~ 35% of the corresponding control values during the last time of the clamp (240 min: lipid infusion; 12.0 ± 1.9 vs. control; 34.7 ± 1.7 mg/kg.min, P<0.0001). At the end of clamp study, the hepatic glucose production (HGP) in controls rats was significantly suppressed (88%) from 19.0 ± 4.5 (basal) to 2.3 ± 0.9 mg/kg.min (P <0.01). The suppressive effect of insulin on HGP was significantly blunted in the lipid-infused rats (200 ~ 240 min: from 18.7 ± 3.0 to 23.2 ± 3.1 mg/kg.min,P < 0.05). The rate of glucose disappearance (GRd) was a slight decrease in the lipid-infused rats compared with controls during the clamp. A lipid infusion of 4 h caused a significant decrease in plasma ghrelin concentration by ~ 83%, when compared to basal levels (490.3 ± 125.6 vs.588.4 ± 118.4 pg/ml, p< 0.05, n = 6), and a significant decrease in plasma ghrelin concentration by ~ 83%, when compared to basal levels (490.3 ± 125.6 vs.588.4 ± 118.4 pg/ml, p< 0.05, n = 6). At the end of euglycemic-hyperinsulinemic clamps plasma ghrelin levels in lipid-infused rats had a slight increase compared with controls (490.3 ± 125.6 vs. 374.6 ± 146.8 pg/ml, p< 0.05).

**Conclusion:** This study demonstrates that during hyperinsulinaemic-euglycaemic clamp, euglycemic hyperinsulinemia decreases circulating ghrelin levels in rats. But an increased circulating FFA's increases slightly plasma ghrelin levels. Our data might be of relevance for disease states involving insulin resistance. At this point of our ongoing study, insulin and FFA induced effects on plasma ghrelin levels have not yet been integrated with data on the metabolic syndrome, because of the limited size of the study cohort.

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## 635

### Insulin resistance is an accelerator, not an initiator, for dietary model of nonalcoholic steatohepatitis

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**Background and aims:** Few would argue that much of the increasing prevalence of nonalcoholic steatohepatitis (NASH) is due to the epidemic of obesity and the metabolic syndrome based on insulin resistance. NASH is a disorder characterized by hepatic steatosis, inflammation, and fibrosis. We have previously shown that hepatic steatosis predicts insulin resistance and increased risks for atherosclerosis, while inflammation and fibrosis predict liver cirrhosis and hepatic failure in patients with nonalcoholic fatty liver disease. However, the pathophysiological links 1) between insulin resistance and the development of NASH, and 2) between steatosis and inflammation/fibrosis remain missing. Here, we induced insulin resistance on the dietary rat models of fatty liver and NASH to clarify the role of insulin resistance in the development of NASH.

**Materials and methods:** Otsuka Long-Evans Tokushima Fatty rats (OLETF), which have been established as an animal model of obese type 2 diabetes, and non-diabetic control Long-Evans Tokushima Otsuka rats (LETO) at 25 to 32 weeks of age were divided into 3 experimental groups and fed for 8 weeks as follows: 1) methionine and choline deficient diet (MCD, n=8), 2) high-fat diet (HF, fat content 81%, n=5), 3) normal chow (fat content 14%, n=5). The livers were examined histologically at 2, 4 and 8 weeks of the experiment, and severity of steatosis, inflammation and fibrosis were each categorized semi-quantitatively. Insulin sensitivity was determined by an oral glucose tolerance tests (GTT) and insulin tolerance tests (ITT) after 8 weeks of the experiment. Expressions of mRNA for transforming growth factor-β1 (TGF-β1) and plasminogen activator-1 (PAI-1) in the liver were estimated by the real-time quantitative RT-PCR.

**Results:** 1) OLETF manifested obesity and hyperinsulinemia at 8 weeks and then developed diabetes at 24 weeks of age. 2) HF increased insulin resistance, resulting in hyperinsulinemia (FPG 153 ± 21 vs. 138 ± 14 mg/dL, NS; fasting serum insulin 307 ± 177 vs. 123 ± 31 pmol/L, $p<0.05$; HF vs. normal chow). 3) OLETF developed fatty liver, which was accelerated with HF. However, even fed with HF, it failed to develop hepatic inflammation and fibrosis. 4) MCD induced NASH in LETO, which was accelerated in OLETF with advanced inflammation and fibrosis. 5) Histologically, MCD induced steatosis alone at 2 weeks, inflammation and fibrosis at 4 weeks, and precirrhosis at 8 weeks in the liver of OLETF. 5) After 8 weeks of the experiment, MCD up-regulated mRNA for TGF-β1 and PAI-1 in the livers of LETO (7.3- and 5.3-fold, respectively, of normal chow, $p<0.01$), and further up-regulated in those of OLETF (28- and 14-fold, respectively, of normal chow, $p<0.0001$).

**Conclusion:** We established the dietary model of fatty liver and NASH with insulin resistance. Steatosis precedes inflammation and fibrosis in the development of NASH. Insulin resistance alone fails to cause NASH, however, it accelerates pathology of NASH. Thus, the insulin resistance-associated initiator that links steatosis to inflammation/fibrosis should be explored.

## 636

### An *in utero* high fat diet results in hypoinsulinaemia in weanling rats

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**Background and aims:** Type 2 diabetes is a multifactorial disease reaching epidemic proportions globally and is characterised by an inability to maintain normal plasma glucose levels. Type 2 diabetes could result either from the inability of the pancreatic β-cell to produce sufficient insulin, in response to high blood glucose concentrations, or the development of insulin resistance. Glucose, which is transported across the β-cell membrane by the glucose transporter, GLUT-2, is known to stimulate insulin secretion. The aim of this study was to determine the effects of an *in utero* high fat diet (HFD) on the concentration of circulating glucose and insulin and on the expression of GLUT-2 in 3-week-old weanlings.

**Materials and methods:** In this study, separate groups (1, 2 and 3) of rats, were exposed to an *in utero* high fat (40% energy as fat) diet for either the first, second or the third week of gestation, followed by a control (10% fat) diet from birth until weaning. Another group was exposed to a maternal high fat diet throughout gestation (Group 4) and yet another throughout gestation and lactation (Group 5). At postnatal day 21, pups were weighed; glucose and insulin concentrations measured, and 6 animals per group were euthanased for immunohistochemistry using antibodies specific for GLUT-2.

**Results:** The weights of progeny of Groups 1–4 (HFD for week 1, 2, 3 or all three) were significantly lower than the control at 3 weeks, while a weight gain was observed in rats exposed to a HFD from conception until postnatal day 21 (Group 5). Although serum insulin concentrations were significantly reduced in all of the experimental groups, including those only exposed to a HFD throughout gestation (Group 4) or throughout both gestation and weaning (Group 5), significant hyperglycaemia was evident only in the offspring exposed to an *in utero* HFD for any single week of gestation (Groups 1–3). It is interesting, however, that the percentage of cells displaying GLUT-2 immunoreactivity was only significantly higher in rats exposed to a HFD for either the last trimester (Group 3) or throughout pregnancy (Group 4), despite the raised glucose levels.

**Conclusion:** Exposure to a HFD at any stage of, or throughout, gestation, was shown to result in significantly reduced weight and insulin levels in the progeny and significantly raised glucose levels in all but the group exposed for the entire gestation. Exposure to a HFD during the third trimester resulted in the most profound effect on all the groups, including significantly raised GLUT-2. The HFD-induced increased demand for insulin and the low circulating insulin levels could ultimately result in β-cell failure and type 2 diabetes.

## 637

### Antioxidant vitamins supplementation rehabilitates glucose homeostasis and ameliorates oxidative status in male offspring of gestational diabetic rats

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**Background and aims:** Our previous investigations have shown that gestational streptozotocin diabetes (GD) induces glucose homeostasis and lipid peroxidation (LP) disturbances in rats offspring. The study was conducted to evaluate the impact of antioxidant vitamins supplementation (AVS) at prepuberty on glucose tolerance, beta-cell function, insulin sensitivity and oxidative status in adult Wistar rats male offspring ($F_1$) of mothers with GD.

**Materials and methods:** The maternal cohort consisted of 20 pregnant rats which were exposed to GD and the controls (C). GD was rendered by streptozotocin injection (45 mg/kg, i.p.) on the 2nd day of pregnancy. $F_1$-rats (n=32) were received AVS (α-tocopherol 50 mg/kg + vitamin C 200 mg/kg b.w./day per os) and placebo within 21st–35th day of age. An i.p. GTT (3g glucose/kg; 0, 30, 60 and 120 min) was performed in $F_1$ at 90 day of age. Fasting blood samples were used for glycemia, plasma insulin (IRI), diene conjugates, malonic dialdehide, α-tocopherol (α-T) and total radical-trapping antioxidant parameter (TRAP) levels determination. Reduced glutathione (GSH) levels and catalase activity (CA) were estimated in liver of $F_1$ at the same age by spectrophotometrically. Homeostasis Model Assessment (HOMA) was used to estimate beta-cell function (HOMA-BCF). Quantitative Insulin Sensitivity Check Index (QUICKI) was used to evaluate insulin sensitivity.

**Results:** Insulin resistance was revealed in sex-maturity $F_1$ male offspring of mothers with GD: HOMA-BCF and AUC/2 h were increased (by 104% and 57% respectively, $p<0.001$ vs C), QUICKI was decreased (0.191 ± 0.003 vs 0.223 ± 0.006 in C, $p<0.001$). LP and CA were enhanced (1.5–2.5 fold, $p<0.001$ and by 25%, $p<0.02$, respectively); α-T, GSH and TRAP were reduced (by 27%, $p<0.01$, 32%, $p<0.001$ and 25%, $p<0.01$, respectively) in GD-$F_1$ as compared to C. AVS improved glucose control in GD-$F_1$: AUC/2 h over GTT was 867.2 ± 28.8 vs 1021.2 ± 18.1 mmol/l/min ($p<0.001$), C: 643.6 ± 7.9 mmol/l/min ($p<0.001$). HOMA-BCF was 858.7 ± 71.6 vs 1192.8 ± 75.4 in GD-$F_1$, $p<0.01$; C: 584.9 ± 30.1, $p<0.01$. AVS attenuated also LP ($p<0.01$ vs GD-$F_1$). The levels of TRAP, CA and QUICKI in antioxidant supplemented GD-$F_1$ did not differ from values obtained in the C, but α-T and GSH were decreased as compared to C ($p<0.05$).

**Conclusion:** Early AVS is possessed of favourable effect on metabolic state in adult male offspring of gestational diabetic rats through improving glycemic control and insulin sensitivity, attenuating lipid peroxidation and increasing antioxidative defence. Using AVS may be beneficial in prevention of metabolic disorders development in offspring of gestational diabetic mothers.

# PS 49
# Adiponectin and associations

## 638

**Evidence for common genetic determinants of adiponectin and HDL-C: the IRAS family study**

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**Background and aims:** Adiponectin appears to play a role in modulating lipid metabolism. Plasma adiponectin were shown to be strongly and positively related to HDL-C level. Both plasma adiponectin and HDL-C have also been shown to be heritable traits.
**Materials and methods:** We examined the hypothesis that both traits share common genetic determinants in Hispanic (HA) and African American (AA) participants in the IRAS Family Study. The analysis included 1539 (58%F) nondiabetic individuals (1027 HA, 512 AA) from 130 families (88 HA, 42 AA). Participants were 40 ± 13 yrs old (mean± SD) with a body mass index (BMI) of 28.6 ± 6.1 kg/m$^2$. Plasma adiponectin was measured by radioimmunoassay (Linco Research, St Charles, MO). Insulin sensitivity ($S_I$) was determined with a frequently sampled intravenous glucose tolerance test with minimal model analysis.
**Results:** Plasma adiponectin was higher in HA than AA before (13.5 ± 6.6 vs. 9.0 ± 4.9 µg/ml; $p<0.01$) and after (13.2 ± 5.8 vs. 9.3 ± 4.0; $p<0.001$) adjusting for age, sex, BMI, and $S_I$. Plasma adiponectin was also approximately 30% higher in women than men of both ethnic groups before and after adjusting for age, BMI, and $S_I$. Plasma adiponectin showed a heritability of 0.83 (0.84 in HA, 0.83 in AA) and HDL-C a heritability of 0.48 (HA: 0.47; AA: 0.53). A variance components approach was used to estimate phenotypic, genetic ($r_G$) and environmental ($r_E$) correlations between HDL-C and plasma adiponectin. The phenotypic correlation was 0.31 ($p<0.01$) adjusting for age, sex, BMI, and $S_I$ (HA: 0.32; AA: 0.29; $p<0.001$ for each). A significant genetic correlation ($r_G = 0.34$; $p<0.001$) was also observed (HA: 0.31, AA:0.40; $p<0.002$ for each) controlling for age, sex, BMI, and $S_I$. The environmental correlation was significant in the combined sample and AA ($r_E = 0.29, 0.41$ respectively; $p<0.03$ for each), but not in HA ($r_E = 0.09$, $p = 0.69$).
**Conclusion:** The phenotypic correlation between plasma adiponectin and HDL-C supports a role for adiponectin in modulating HDL-C level and pathogenesis of dyslipidemia. Nonetheless, plasma adiponectin and HDL-C also appear to share genetic determinants independent of obesity and insulin resistance. This finding could be attributed to a pleiotropic gene (s) that affect both traits. Thus, the association between plasma adiponectin and HDL-C has both environmental (obesity and insulin resistance-related) and genetic components.

## 639

**Ethnic differences in adiponectin levels, insulin sensitivity and beta-cell function in healthy, glucose-tolerant subjects**

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**Background and aims:** Ethnic differences in the prevalence of type 2 diabetes (T2D) and cardiovascular disease (CVD) are well-documented. Insulin resistance is thought to be the unifying feature of the metabolic syndrome, which is intimately associated with both T2D and CVD. Studies have suggested an important role of adiponectin in insulin resistance. Here, we investigated the effect of ethnicity on adiponectin levels, insulin release and insulin action.
**Materials and methods:** Thirty-six healthy, glucose-tolerant subjects of 3 different ethnic group (Caucasian [Cau], Chinese [Chi] and Indian [Ind]), closely matched for their age, body mass index (BMI) and physical activity, were studied. Anthropometry, body composition (bioelectric impedance), and fasting adiponectin levels were evaluated. All subjects underwent oral glucose tolerance test (OGTT) and frequently-sampled intravenous glucose tolerance test (FSIVGTT).
**Results:** There was no significant difference in age, BMI, waist-hip ratio, percentage body fat and fasting triglycerides. Both Indians and Chinese have significantly higher fasting total cholesterol (Cau 4.38 ± 0.53, Chi 5.02 ± 0.77, Ind 5.29 ± 1.13 mmol/L; p=0.032, ANOVA), LDL-cholesterol (Cau 2.60 ± 0.46, Chi 3.24 ± 0.65, Ind 3.43 ± 1.15 mmol/L; p=0.028), 1$^{st}$-phase insulin (Cau 35.28 ± 20.58, Chi 67.05 ± 39.91, Ind 74.27 ± 61.68 mU/L;

p=0.013) and 2nd-phase insulin (Cau 3.77 ± 1.91, Chi 7.58 ± 2.09, Ind 11.07 ± 8.51 mU/L; p<0.001), and lower insulin sensitivity (Cau 10.1 ± 5.0, Chi 4.4 ± 2.0, Ind 4.9 ± 2.4 $10^{-4}$x (min × mU/L)$^{-1}$; p<0.001) and fasting adiponectin levels (Cau 12.06 ± 4.10, Chi 8.05 ± 2.10, Ind 9.06 ± 3.37 µg/mL; p<0.015) compared to Caucasians. Stepwise regression analysis of adiponectin levels demonstrated that only fasting insulin levels and ethnicity are significant (p=0.002 & p=0.019 respectively, $R^2$ = 0.411) among variables BMI, waist-hip ratio, systolic and diastolic BP, triglycerides and HDL-cholesterol, percentage body fat, insulin sensitivity, 1st- and 2nd-phase insulin.

**Conclusion:** This study demonstrated that there is significant insulin resistance with compensatory insulin response in healthy, glucose-tolerant Indians and Chinese compared to Caucasians. We have shown that fasting insulin and ethnicity have independent effects in determining the fasting adiponectin levels in healthy subjects.

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### Higher molecular weight adiponectin not total is a better correlate of glucose intolerance

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**Background and aims:** Adiponectin (Acrp30) is an adipocytokine which is implicated with mediating systemic insulin sensitivity through action on liver and muscle. It is well established that total systemic Acrp30 is reduced with increased adiposity as well as type 2 diabetes. However it is apparent that the native protein circulates in serum as a lower molecular weight (LMW) hexamer and as larger multimeric structures of high molecular weight (HMW). The HMW form is reduced in type 2 diabetic subjects and db/db mice although it is still unclear which form of the protein is more important for whole body insulin sensitivity in humans. Therefore in this study, we address the clinical significance of each form of the protein with respect to glucose tolerance and the effect of several clinical parameters on HMW Acrp30.

**Materials and methods:** Serum was obtained from 30 indo Asian subjects (8 female (BMI 25.06 ± 1.82; age 44 ± 14.1); 22 Male (BMI 26.3 ± 3.2; age 51.5 ± 11.2) who had undertaken a 2 hr oral glucose tolerance test. An aliquot of serum was fractionated using velocity sedimentation followed by reducing SDS-PAGE. Western blots were probed for Acrp30 and the percent of HMW Acrp30 over total (Sa) was calculated from densitometry readings. Total Acrp30 was measured using a standard RIA, leptin and C reactive protein (crp) were determined using ELISA.

**Results:** The males had Sa levels that were ~34% (p=0.011) less than females. In addition to this diabetic males had ~38% (p=0.0098) less Sa levels compared to healthy male subjects. Further analysis of the male cohort demonstrated that both total acrp30 (r=0.62; p=0.017) and age (r=0.53; p=0.011) showed a positive association with Sa. Interestingly Sa was inversely correlated with 2 hr glucose levels (r=–0.57; p=0.0050), which was not observed with total acrp30. A negative correlation of Sa with BMI, crp and leptin was apparent however this did not reach significance.

**Conclusion:** This study underlines the importance of this measurement as a better determinant of glucose intolerance compared to measurements of total Acrp30. This supports the emerging hypothesis that HMW Acrp30 is the active form of the protein.

*Supported by: EFSD Travel Fellowship*

## 641

### Association of adiponectin receptor (AdipoR1 and AdipoR2) mRNA content of human myotubes with metabolic parameters of myotube donors

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**Background and aims:** The recently identified adiponectin receptors, AdipoR1 and AdipoR2, are thought to mediate adiponectin's insulin-sensitizing, anti-inflammatory, and atheroprotective effects. The aim of this study was to determine whether AdipoR mRNA expression of in vitro differentiated skeletal muscle cells (myotubes) reflects insulin sensitivity of the myotube donors.

**Materials and methods:** mRNA expression of AdipoR1 and AdipoR2 in human myotubes from 40 healthy normal glucose-tolerant donors was quantified by real-time RT-PCR. The donors were metabolically characterized by OGTT and hyperinsulinemic-euglycemic clamp. The relationship between myotube AdipoR mRNA content and in vivo parameters of glucose and lipid metabolism was studied by correlational analysis.

**Results:** Human myotubes expressed 1.8-fold more AdipoR1 than AdipoR2 mRNA (588 ± 35 vs 321 ± 39 fg/µg total RNA). In addition, the mRNA levels of both receptors correlated with each other (r=0.45, p<0.01, N=40). AdipoR1 mRNA expression was positively correlated with serum concentrations of insulin and C-peptide, with 1st-phase insulin secretion as well as with plasma concentrations of triglycerides and cholesterol. These correlations persisted after adjustment for sex, age, waist-hip ratio, and percentage of body fat. By contrast, AdipoR2 mRNA levels were only correlated with plasma triglycerides. In multivariate linear regression models, mRNA expression of AdipoR1, but not AdipoR2, turned out to be a determinant of 1st-phase insulin secretion independent of insulin sensitivity and body fat mass. Finally, no direct impact of insulin on AdipoR1 mRNA expression in human and murine myotubes was detectable in vitro.

**Conclusion:** We show here that myotube AdipoR mRNA levels associate with distinct metabolic functions, but not with insulin sensitivity. Surprisingly, mRNA expression of AdipoR1 is related to insulin secretion. Since insulin does not modulate AdipoR1 mRNA expression in myotubes, the molecular nature of this link between muscular AdipoR1 expression and pancreatic insulin secretion is still unclear and awaits further studies.

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## 642

### Changes in adiponectin level in Type 1 diabetic children

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**Background and aims:** In the pathogenesis of type 1 diabetes (T1D), near to insulin secretion deficiency as a result of chronic β-cells destruction, the other disorders e. g. in adipose cells are also observed. One of the most important adipocyte-expressed cytokine in human serum is adiponectin (adipoQ).

The aim of this study was to determine an association between adiponectin serum level and clinical characteristics of type 1 diabetes in children.

**Materials and methods:** For this purpose 129 type 1 diabetic patients (50 female and 79 male, mean age = 9.7 ± 4.1 years) and 50 healthy children as a control group (18 female and 32 male, mean age = 11.4 ± 4.7 years) were examined. The levels of adipoQ were measured by radioimmunoassay at the onset and in 6th, 24th, 36th, 48th month after diagnosis. Among the clinical features: insulin requirement (U/kg/24 h), HbA1 c levels, z-score as BMI normalized by age and sex, fasting glycaemia and clinical remission were considered.

**Results:** The adiponectin level decreased during the T1D: at the onset –19.0 (12.1–28.7)µg/ml, in 6 month-16.7 (quartiles: 9.9–22.5)µg/ml, after 24 months –14.3 (8.5–20.8)µg /ml, after 36 months –10.8 (8.5–15.4)µg/ml and after 48 months –9.6 (7.1–12.4)µg/ml (p<0.001). Moreover, a correlation between adipoQ levels at the onset and in the 6th month of the disease duration (r=0.25, p<0.03) was found. The adiponectin level in the 6th month of diabetes was negatively correlated with age at the onset (r =–0.23, p<0.008). In the 48th month of the disease duration the adiponectin level was statistically lower in the patients than in the control group (10,45 ± 7,42 µg/ml vs 15,47 ± 5,3 µg/ml, p<0,007). No differences in adiponectin level were found as gender and other clinical parameters were concerned.

**Conclusion:** Concluding, it was difference in adiponectin level between type 1 diabetic patients and healthy children. The level of adiponectin decreased during the type 1 diabetes.

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## 643

### Adiponectin levels are reduced in HIV infection and after treatment and correlate with VLDL and IDL apolipoprotein B fractional catabolic rate

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**Background and aims:** Adiponectin levels have been shown to be related to lipodystrophy, insulin resistance and lipid levels in HIV patients. No previous studies have investigated the relationship between adiponectin and lipoprotein kinetics.

**Materials and methods:** VLDL and IDL apolipoproteinB (apoB) absolute secretion rate (ASR) and fractional catabolic rate (FCR) were measured with a 9-hour infusion of $^{13}$C-leucine in 9 HIV negative control subjects,

15 treatment naïve HIV positive patients (TN) and 40 HIV positive patients receiving highly active antiretroviral therapy (HAART). Insulin resistance was calculated by the homeostatic model (HOMA) and body fat was measured by a whole body DEXA scan. Plasma triglyceride, total cholesterol and adiponectin were also measured. Results are expressed as mean±SEM.
**Results:** Age (Controls 34 ± 4, TN 38 ± 3, HAART 40 ± 2yr) and BMI (Controls 23.1 ± 1.2, TN 24.4 ± 0.7, HAART 23.6 ± 0.5 kg/m$^2$) were not significantly different in the 3 groups. Patients in the HAART group had been receiving treatment for 38 ± 3 months. Adiponectin levels were significantly lower in the HIV treatment naïve patients (6.3 ± 0.8 µg/ml, $p<0.02$) and the HAART group (5.3 ± 0.5 µg/ml, $p<0.001$) compared to the Controls (11.1 ± 1.6 µg/ml). Plasma triglyceride was significantly higher in the HAART group (1.94 ± 0.19 mmol/l) than the Controls (1.1 ± 0.15 mmol/l, $p<0.05$). Trunk fat, insulin resistance, total cholesterol, VLDL ASR and IDL ASR were not significantly different in the 3 groups. Peripheral fat corrected for BMI was significantly lower in HAART patients (234 ± 19g/BMI) than in the Controls (390 ± 42, $p< 0.001$) and TN patients (313 ± 29, $p<0.04$). VLDL FCR (6.3 ± 0.4pools/d) was significantly lower in HAART than Controls (13.4 ± 1.7, $p<0.001$). IDL FCR (4.0 ± 0.4pools/d) was also significantly lower in HAART than Controls (9.6 ± 1.4, $p<0.001$). In the HIV patients serum adiponectin correlated negatively with HOMA ($r = -0.33$, $p<0.02$) and plasma triglyceride ($r=0.50$, $p<0.0002$) and positively with VLDL FCR ($r = 0.51$, $p<0.0001$), IDL FCR ($r = 0.47$, $p<0.0001$) and peripheral fat/BMI ($r = 0.55$, $p<0.0001$) but not VLDL or IDL ASR.
**Conclusion:** HIV infection significantly lowers adiponectin levels and levels remain low with treatment. The strong correlation between adiponectin and VLDL and IDL FCR suggests that this hormone may have a direct effect on lipoprotein metabolism possibly via lipoprotein lipase.
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# PS 50
# Adiponectin and interventions

## 644

**Basal and exercise-induced adipokine release from human adipose tissue *in vivo***
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**Background and aims:** Adipokines [e.g. adiponectin, leptin, interleukin(IL)-6, IL-8, tumor necrosis factor (TNF)-α and monocytes chemottracant protein (MCP)-1] are released from human adipose tissue *in vitro* and circulating levels are found to be associated with obesity-related disease. Adipokine release from the abdominal, subcutaneous adipose tissue (AT) depots was studied during basal conditions and during sub-maximal exercise [e.g. 60% of maximal oxygen consumption].
**Materials and methods:** In healthy subjects, adipokine release was estimated using an arterial-venous difference technique by which blood simultaneously was extracted from a catheterised artery and a vein draining the abdominal subcutaneous AT depot. AT blood flow was measured by a $^{133}$Xe-washout technique.
**Results:** Venous concentration of IL-6, IL-8 and leptin were 75% ($p<0.01$), 30% ($p<0.05$) and 37% ($p<0.05$) higher than arterial, respectively. Surprisingly, no net release of adiponectin was observed. Exercise induced a 4-fold higher IL-8 release compared to basal IL-8 release ($p < 0.05$). In contrast, exercise did not affect adiponectin or leptin concentrations. Neither basal nor exercise-induced release of TNF-α or MCP-1 was observed.
**Conclusion:** The present data demonstrate that there is a net release of IL-6, IL-8 and leptin from the subcutaneous abdominal AT depot as well as an exercise-induced IL-8 release. The results in the present paper are in line with the hypothesis that AT-derived proteins are released to the circulation with potential effects on whole-body metabolism.
*Supported by: The Danish Medical Research Council*

## 645

**Plasma adiponectin decreases during 24 hour moderate hyperinsulinemia but not after inhibition of lipolysis by Acipimox**
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**Background and aims:** Adiponectin has anti-diabetic and anti-atherosclerotic properties and its plasma level is negatively correlated with insulin resistance. Adiponectin tissue expression is upregulated by PPARγ agonist treatment and may be affected by insulin. We tested the hypothesis that exogenous insulin affects plasma adiponectin in vivo, and examined whether a possible effect of insulin on adiponectin is attributable to inhibition of lipolysis.
**Materials and methods:** The effect of insulin infusion on plasma adiponectin (Linco enzyme-linked immunosorbent assay) was evaluated during a 24 h moderately hyperinsulinemic clamp (30 mU/kg/h) in 8 male type 2 diabetic patients (age 59 ± 7 yr, body mass index 24.0 ± 2.4 kg/m$^2$; isoglycemic; mean blood glucose: 7.9 mmol/l) and in 8 healthy men (age 51 ± 9 yr, body mass index 27.8 ± 1.5 kg/m$^2$; euglycemic; mean blood glucose: 4.3 mmol/l). On a separate day, Acipimox (250 mg/4 h for 24 h) was administered to inhibit lipolysis. Insulin and Acipimox were administered in random order with 1 week between study days.
**Results:** In type 2 diabetic patients, insulin infusion decreased plasma adiponectin from 7.74 ± 2.53 mg/l to 6.76 ± 2.41 mg/l after 24 h ($p<0.05$). In healthy subjects, the changes in plasma adiponectin were not significant (8.10 ± 2.76 and 7.55 ± 2.41 mg/l at baseline and after 24 h insulin, respectively). Plasma adiponectin did not decrease after 24 h Acipimox administration in either group (type 2 diabetic patients: 6.84 ± 2.19 and 6.54 ± 2.93 mg/l at baseline and at after 24 h, respectively (NS); healthy subjects; 7.35 ± 2.52 and 8.31 ± 3.37 mg/l, at baseline and after 24 h, respectively (NS). Plasma free fatty acids decreased during insulin infusion ($p < 0.01$ for both groups) as well as in response to Acipimox ($p < 0.02$ for healthy subjects; $p < 0.01$ type 2 diabetic patients). Plasma insulin did not change in either group after Acipimox.
**Conclusion:** Plasma adiponectin is modestly decreased during 24 h insulin infusion. It is unlikely that this response to exogenous insulin is due to inhibition of lipolysis, as evidenced by the lack of change in plasma adiponectin after Acipimox.

## 646

**Impact of an enrichment of the diet in ω-3 fatty acids on glucose metabolism and plasma adiponectin concentration**

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**Background and aims:** Cardiovascular benefits conferred by long-chain ω-3 fatty acids have been well established but the molecular mechanisms involved remain poorly understood. Insulin-resistance is associated with a low plasma concentration of adiponectin and an enhanced risk of cardiovascular disease. The aim of our study was to investigate in healthy non-diabetic subjects the impact of an increased consumption of foods rich in ω-3 fatty acids on glucose metabolism and adipocytokine concentration involved in insulin-resistance (adiponectin and TNF-α).
**Materials and methods:** Twenty healthy non-diabetic subjects were following dietary recommendations with the purpose to increase ω-3 fatty acids intake without increasing total energy intake (consumption of fat fish at least 3 times per week, 20g of rapeseed oil per day). The measure of phospholipid content of erythrocyte's membrane was used to check the adherence to the diet. Insulin sensitivity was assessed by hyperinsulinemic-euglycemic clamp, glucose and lipid oxidation were assessed by indirect calorimetry.
**Results:** After 10 weeks of dietary intervention, the ω-6/ω-3 fatty acids intake ratio was significantly reduced without changes in body weight or waist circumference. There were no significant changes in lipid parameters but a trend for an increase in HDL-c level. Insulin sensitivity did not change after the dietary intervention but there was an increase in lipid oxidation in patients. Plasma concentration of adiponectin was significantly increased ($6.5 \pm 0.7$ vs $7.6 \pm 0.1$, $p=0.01$) whereas level of TNFα was reduced ($2.2 \pm 0.3$ vs $1.5 \pm 0.3$, $p=0.06$). In contrast, plasma leptin ($9.2 \pm 2.6$ vs $8.4 \pm 2.8$), IL6 ($1.28 \pm 0.23$ vs $1.00 \pm 0.17$) and ultra-sensitive CRP levels did not change after enrichment of the diet in ω-3 fatty acids.
**Conclusion:** These findings show that qualitative changes in the pattern of fatty acids intake with increasing ω-3 fatty acids consumption is associated with an increase in adiponectin concentration and reduced TNFα level. These data suggest the interest of increasing ω-3 fatty acids consumption, without using high-dose pharmacological supplementation, in cardiovascular and metabolic prevention.

## 647

**Adiponectin concentrations in offspring exposed to maternal protein restriction**

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**Background and Aims:** SGA children who experience a period of accelerated growth are at a greater risk for the development of insulin resistance and have been shown to have reduced adiponectin concentrations. Adiponectin is an adipocytokine found to have an inverse relationship with insulin resistance and adiposity. It has also been found to reduce the expression of genes involved in hepatic glucose production. Offspring from the low protein animal model of maternal undernutrition (a model of fetal undernutrition) have increased whole body insulin sensitivity at a young age with an increased expression of phosphoenolpyruvate carboxykinase (PEPCK) which is a rate-limiting enzyme involved in hepatic glucose output. The aim of this study was to measure adiponectin concentrations in offspring from the low protein model of maternal undernutrition and to determine a role, if any, for this adipokine in the altered insulin sensitivity which has been reported in this model.
**Materials and methods:** Dams were fed either a control (20% protein) or a low protein (8% protein) diet throughout gestation and lactation. Offspring were studied at 26 days (weaning) and at 15 months after maintenance on a controlled energy intake of the control diet from weaning. Adiponectin concentrations (ng/ml), adiposity (white adipose tissue weights (g)/body weight (g)) and the glucose to insulin ratio ($mM/ngml^{-1}$) were measured at each age. Hepatic PEPCK expression was also measured at 26 days of age using reverse transcriptase PCR.
**Results:** At 26 days LP offspring demonstrated increased adiponectin concentrations (CON: $4.8 \pm 0.2$ vs LP: $6.0 \pm 0.32$, $p = 0.01$) (ng/ml), no change in adiposity ($p = 0.37$) and a greater ratio of glucose to insulin than CON offspring (CON: $2.9 \pm 0.3$ vs LP: $13.3 \pm 1.2$, $p < 0.0001$). A positive correlation between adiponectin and the glucose to insulin ratio was also evident at this age ($p < 0.03$, $R^2 = 0.3$). By 15 months of age there was no effect of maternal diet on adiponectin concentrations (CON: $6.3 \pm 0.2$ vs LP: $6.4 \pm 0.4$, $p = 0.9$), adiposity ($p = 0.2$) nor the glucose to insulin ratio ($p = 0.8$). At 26 days a significant negative correlation was found between adiponectin and hepatic PEPCK expression ($p = 0.002$, $R^2 = -0.6$) however no significant difference in PEPCK expression was evident between the groups at this age (CON: $0.64 \pm 0.1$, LP: $0.50 \pm 0.05$, $p = 0.08$).
**Conclusion:** This study has demonstrated adiponectin to be a good indicator of an altered glucose to insulin ratio (a measure of insulin sensitivity). The increased adiponectin in the low protein offspring may demonstrate an important role for adiponectin in whole body insulin sensitivity in the low protein offspring at an early age. The lack of a significant reduction in PEPCK expression in the presence of increased adiponectin concentrations may indicate that this adipokine has no major effect on the expression of this controlling enzyme but an effect on other aspects of the enhancement of insulin sensitivity in LP offspring.

## 648

**Adiponectin receptor expression is not regulated by troglitazone in *in vitro* differentiated human myotubes**

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**Background and aims:** Thiazolidinediones (TZD) are high affinity ligands of the transcription factor PPARγ and improve insulin sensitivity in insulin-responsive tissues. One possible molecular mechanism of the insulin-sensitizing effect by TZD is the modulation of the expression of adipokines and their receptors. Recent data show an induction of adiponectin receptor 2 by PPARγ agonists in human macrophages.
In this study, we examined the regulatory effects of troglitazone on the expression of adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) in human skeletal muscle cells, the cell-type most relevant for whole-body insulin sensitivity.
**Materials and methods:** In vitro differentiated human myotubes were treated with troglitazone during ($n = 5$) or after ($n = 8$) differentiation.
The expression levels of PPARγ, AdipoR1, AdipoR2, Δ-6 desaturase mRNA and 28SrRNA were quantified by RT-PCR.
**Results:** Effectiveness of troglitazone was demonstrated by changes of expression levels of PPARγ and Δ-6 desaturase. Treatment of cultured human myotubes during or after differentiation with troglitazone resulted in a significant increase in PPARγ mRNA ($p < 0.05$) and significant reduction of Δ-6 desaturase mRNA ($p < 0.05$).
In order to investigate the regulatory influence of TZD on adiponectin receptors, myotubes were treated with 10 μM troglitazone for 20 h after differentiation. This resulted in a non-significant reduction of AdipoR1 gene expression by 18% and a non-significant reduction of AdipoR2 gene expression by 24% ($n = 8$).
Analogously, myotubes treated with 11.5 μM troglitazone for 5 days during the differentiation period showed a non-significant reduction of AdipoR1 gene expression by 32% and a non-significant reduction of AdipoR2 gene expression by 42% ($n = 5$).
**Conclusion:** Our data show no significant regulation of AdipoR1 and AdipoR2 by troglitazone in human myotubes. Therefore, we conclude that induction of adiponectin receptor expression in human skeletal muscle cells is not involved in the insulin-sensitizing effects of TZD.

# PS 51
# Nervous system and metabolism

## 649

**Human insulin sensitivity regulation by cholinergic pathway**

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**Background and aims:** The liver modulates insulin sensitivity by the release of the Hepatic Insulin Sensitizing Substance (HISS) that accounts for 55% of peripheral insulin action. HISS release in response to insulin is blocked by interfering with the hepatic parasympathetic nerves in any of a number of ways including physiologically, pharmacologically, surgically and by pathology. 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. Our hypothesis is that the existence of a HISS-dependent component of insulin action is regulated by a cholinergic mechanism in humans.
**Materials and methods:** Healthy male subjects (36.6 ± 3.7 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 transient hyperinsulinemic euglycemic clamp technique to determine the glucose disposal produced by a bolus of 50 mU/kg 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 performed a euglycemic clamp in two different days; intravenous infusions of either 0.5 mg atropine (a low therapeutic dose 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 and atropine group. In the control group (saline infusion) the glucose disposal was 539.4 ± 81.4 mg glucose/kg (n=5). In the atropine group, the glucose disposal decreased to 391.3 ± 52.0 mg glucose/kg (n=5), $p<0.05$.
**Conclusion:** Our data support the hypothesis that intravenous atropine administration results in HISS-dependent decreased insulin sensitivity due to blockade of the hepatic parasympathetic nerves, a result similar to that obtained with laboratory animals.
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## 650

**Does aging alter the hepatic parasympathetic nerve-dependent component of insulin sensitivity differently according to gender?**

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**Background and aims:** Although we found no gender difference on glucose tolerance at a younger age, some authors report the existence of gender differences in the age-related decrease in insulin sensitivity. Moreover, previous studies have showed that insulin-stimulated glucose uptake has two components: a Hepatic Parasympathetic Nerve (HPN) -dependent component, that leads, through a neurohumoral signal, to the sensitisation of skeletal muscle to insulin, and a HPN-independent component, the direct action of insulin on target tissues. The HPN-dependent component can be interrupted by ablation of the HPN or blockade of the hepatic muscarinic receptors. In this study, we tested the hypothesis that even though the decrease in insulin action with aging is dependent on the HPN-dependent component of insulin sensitivity, it is not influenced by gender.
**Materials and methods:** Insulin sensitivity was determined at 9 and 52 weeks of age, on male and female Wistar rats, by a transient euglycemic clamp performed after the administration of a bolus of insulin (50 mU/kg). The HPN-independent component was assessed by blocking the HPN-dependent component with atropine (3 mU/kg), a muscarinic antagonist. The HPN-dependent component was quantified by subtracting the insulin action obtained after atropine from the control one.
**Results:** Insulin sensitivity decreased from 9 to 52 weeks similarly in male (299.5 ± 20.8 to 166.4 ± 13.1 mg glucose/kg bw, $p<0.001$) and female (265.7 ± 29.8 to 175.5 ± 20.1 mg glucose/kg bw, $p<0.05$). The HPN-independent component remained unchanged with age and gender (male: 132.1 ± 19.5 to 133.8 ± 9.8; female: 127.2 ± 10.5 to 129.7 ± 12.3 mg glucose/kg bw). Moreover, the HPN-dependent component was decreased similarly in both sexes (male: 167.3 ± 21.0 to 32.7 ± 5.7, $p<0.001$; female: 138.6 ± 29.0 to 45.8 ± 13.0 mg glucose/kg bw, $p<0.001$).
**Conclusion:** This study supports our hypothesis that aging-related insulin resistance develops similarly in male and female Wistar rats, due to a dysfunction of the HISS-dependent component.
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## 651

**Effect of Adrenomedullin on insulin sensitivity of normal Wistar rats in vivo**

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**Objectives:** To Investigate the effect of Adrenomedullin (AM) on insulin sensitivity of normal Wistar rats.
**Materials and Methods:** 20 normal male Wistar rats of 2~3 months old weighing 290~326g were divided into 4 groups (groupA~D) randomly to the infusion rates of AM during clamp studies.The infusion rates of AM were 0,0.05,0.2,1.0 μg/kg/min, respectively.The left carotid artery was cannulated to allow sampling and the right jugular vein was cannulated for infusion.All rats were performed heperinsulinemic-euglycemic clamp in combination with isotope dilution technique 4 days after the surgery.The glucose disposal rate (GDR) and hepatic glucose output (HGO) were calculated by the plasma 3-$^3$H-glucose specific activity.
**Results:** After AM infusion,the insulin-mediated glucose disposal reduced in a dose-dependant tendency manner (the insulin stimulated GDR of group A~D were 29.99 ± 6.73, 26.82 ± 5.56, 23.47 ± 8.03, 14.69 ± 2.03 mg/kg/min, respectively), but significant difference only showed between group D and group A (control group) ($P<0.01$),while AM infusion at a rate of 0.05 or 0.2 μg/kg/min fail to affect insulin stimulated glucose disposal significantly, which means that only high dose of AM infusion can induce a marked state of insulin resistance in normal Wistar rats.Increase of hepatic glucose output was not observed by AM infusion at any rate.
**Conclusions:** High dose AM could cause a marked state of insulin resistance when infused in vivo into normal Wistar rats, which mainly was characterized by inhibition of the effect of insulin to simulate peripheral glucose metabolism.It indicated that Hyperadrenomedullinemia at higher adrenomedullin level may produce insulin resistance.

## 652

**Independent evolutive patterns of free fatty acid and lipid oxidation, insulin resistance and sympathetic activity during gastric bypass-induced body weight loss**

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**Background and aims:** Obesity is characterized by elevated plasma Free Fatty Acid (FFA) and lipid oxidation rate as well as high insulin resistance and sympathetic activity. It is also well known that body weight reduction leads to an improvement of all these parameters. This study was aimed at evaluating the evolutive patterns of FFA and lipid oxidation, insulin resistance and sympathetic activity along the gastric bypass-induced body weight loss.
**Materials and methods:** Ten morbid obese patients were submitted to a hyperinsulinemic clamp as well as to a 24-hour ECG recording (Holter) before, 3 and 12 months after gastric bypass. During the clamp, together with glucose uptake, substrates oxidation rate (indirect calorimetry) and plasma free fatty acid were measured.
**Results:** During the clamp, both before and 3 months after surgery, plasma FFA and lipid oxidation rate showed a divergent pattern: in fact, while FFA were substantially inhibited by hyperinsulinemia, lipid oxidation was only marginally reduced. Twelve months after surgery, both Free Fatty Acid and lipid oxidation rate were markedly lowered during hyperinsulinemic clamp. As compared to pre-operative conditions, glucose uptake progres-

sively increased 3 and 12 months after surgery ($p < 0.005$ for both). Power spectral analysis of Heart Rate Variability showed a net sympathetic prevalence in pre-operative conditions; sympathetic activity was markedly reduced 3 months after surgery ($p < 0.0001$) and tended to return to higher values, 12 months after surgery ($p < 0.01$ vs 3 months values).
**Conclusion:** 1) Lipid oxidation is only partially supported by circulating FFA : the persistence of high lipid oxidation rate in presence of low plasma FFA suggests that lipid oxidation is fed by intra-myocellular triglyceride. 2) glucose uptake and sympathetic activity do not evolve in parallel during gastric bypass-induced body weight loss. This suggests that factors other than the improvement of insulin resistance contribute to the control of sympathetic activity.
*Supported by: Swiss National Fund for Scientific Research*

## 653

**Amylin inhibition of ghrelin secretion depends upon an intact *area postrema***
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**Background and aims:** Ghrelin, a 27/28aa n-octanoylated peptide secreted from the stomach, stimulates growth hormone (GH) secretion via agonism at the GH secretagogue receptor. Ghrelin secretion is high in conditions of β-cell insufficiency, such as insulinopenic diabetes and Prader-Willi syndrome, and insulin can partly suppress ghrelin secretion. Amylin, also deficient in insulinopenic diabetes, acts at the *area postrema* (AP) a circumventricular organ that is densely populated with amylin receptors. We tested the idea that amylin regulates ghrelin secretion via the *area postrema*.
**Materials and methods:** Following an 18-hour fast, tails of conscious lightly-restrained Sprague Dawley® rats were locally anesthetized for serial sampling for active and total ghrelin (Linco RIA kits) at 0, 10, 20, 30, 60, 90 and 120 min after subcutaneous injection of saline or rat amylin in doses of 1, 10, 30 or 100 μg/kg (n=5, 4, 5, 5, 4, respectively). Fasted rats with localized lesions of AP made by vacuum aspiration 3 weeks before experiments and sham operated rats were injected subcutaneously with amylin (30 μg/kg) or saline (n=4/group). To test for effects of endogenous amylin, samples were also taken from fed rats after an intravenous injection of the selective amylin antagonist, AC187 alone (3 mg) or saline (n=12, 11, respectively).
**Results:** Amylin dose-dependently and potently reduced concentrations of total ghrelin (ED50=2.3 μg/kg; ΔAUC90 min) and reduced active ghrelin by up to 49% (0–90 min mean; $P<0.02$ ANOVA). Twenty minutes after a 30 μg/kg subcutaneous amylin injection in Sham rats, active ghrelin concentration decreased by 31.3 ± 3.3%, while active ghrelin concentration in saline controls increased by 23.5 ± 22.1% ($P<0.05$). In AP-lesioned rats, there was no significant difference in ghrelin concentration in those receiving amylin *vs* those receiving saline (increase of 13.7 ± 17.6% *vs* increase of 49.6 ± 21.0%; $P = 0.24$). Five minutes after administration of AC187 in fed animals, active ghrelin concentration increased by 16.3 ± 4.0%, as compared to a 1.4 ± 6.4% decrease in saline controls ($P<0.03$).
**Conclusion:** Results are consistent with amylin being an endogenous inhibitor of ghrelin secretion. Elevated ghrelin (and thence, GH secretion) reported during β-cell deficiency may thereby be at least partly attributable to a lack of amylinergic suppression. The effect of *area postrema* lesions to annul amylin inhibition of ghrelin secretion indicates that amylin acts via this brain stem structure to evoke this effect.

## 654

**Declarative and working memory deficits in middle-aged, insulin resistant men**
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**Background and aims:** Insulin not only plays a role in peripheral glucose regulation but also has an impact on central nervous system function. Recent data suggests that, by acting in the CNS, insulin may modulate cognitive activity, in particular learning and memory. Indeed, hyperinsulinemia has been found to be associated with general cognitive decline in an elderly, non-diabetic population whilst in Alzheimer's disease patients, defects in insulin action have been reported both in the periphery and brain. This study examines whether deficits in memory are associated with insulin resistance (IR) in a middle-aged non-diabetic population.
**Materials and methods:** Participants were non-diabetic men, aged 40–66 years ($N=44$). Insulin resistance (IR) was determined using the Homeostasis Model of Assessment. Low ($n=23$) and high ($n=21$) IR groups were defined as HOMA-IR <1.3 and HOMA-IR >2.6, respectively. Both groups were age and education matched. The Californian Verbal Learning Test (CVLT) was used to assess declarative memory. Four tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB) were used to assess different aspects of non-verbal memory: Delayed Matching to Sample (DMS), Pattern Recognition Memory (PRM), Paired Associate Learning (PAL), and Spatial Working Memory (SWM). The National Adult Reading Test (NART) was used as a proxy measure of IQ.
**Results:** The mean age for low and high IR groups was 51.7 and 53.9 years, respectively. CVLT results revealed significant differences between the two conditions. The high IR group remembered significantly less words than the low IR group after either a short or long delay between encoding and recall. However, immediate recall and short delay cued recall did not differ significantly. See Table 1 for results.

| | Low IR ($n=23$) | High IR ($n=21$) | $p$ - level |
|---|---|---|---|
| Immediate recall trial 1 | 6.7 | 6.3 | *ns* |
| Short delay free recall | 11.6 | 9.5 | <0.02 |
| Short delay cued recall | 12 | 11.3 | *ns* |
| Long delay free recall | 12 | 10.3 | <0.03 |
| Long delay cued recall | 12.5 | 10.8 | <0.03 |

*Table 1:* Mean number of correctly recalled words on the CVLT for low and high IR groups. *Ns* = non-significant at the 5% level. Age, years of education and NART score were included as covariates if significant at the $p<0.1$ level. Performance on CANTAB tests which assess working memory, DMS and SWM, differed significantly between the two groups. In the DMS task, the low IR group gave significantly more correct responses than the high IR group (mean 36.4 vs 35, $p < .05$), whilst for the SWM test, there was a tendency for the high IR group to make more errors than the low IR group (mean 13.4 vs. 6.7, $p < 0.07$). Performance on both PRM and PAL was not affected by condition (both $F < 1$).
**Conclusions:** Middle-aged, non-diabetic men with high levels of IR show impaired performance on tests of both declarative and working memory. The study suggests that the association between insulin resistance and memory deficits, previously only observed in an elderly population, may be a more general finding.

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# Obesity: treatment

## 655

**Effect of rosiglitazone and metformin on muscle and hepatic lipid parameters in male obese Zucker diabetic fatty rats**

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**Background and aims:** Insulin resistance is associated with increased levels of circulating lipids as well as intramyocellular (IMCL) and hepatocellular (HepCL) lipids. Long Chain Acyl-CoA´s (LCACoA) are discussed to be a causative factor in the development of insulin resistance. Rosiglitazone (RGZ) is an antidiabetic agent that improves insulin sensitivity by activation of the peroxisome proliferator-activated receptor-γ (PPARγ). Metformin (MET) decreases blood glucose levels by reducing the endogenous glucose production. The male obese Zucker Diabetic Fatty (ZDF) rat is an animal model for insulin resistance. These animals spontaneously develop a manifest diabetic state from the age of 10 wks. Aim of this study was to investigate effects of treatment of insulin resistant rats with the above-mentioned antidiabetic agents on tissue lipid parameters and metabolic serum parameters.
**Materials and methods:** Male obese ZDF rats were treated with RGZ (3 mg/kg/day orally) or MET (400 mg/kg/day orally) starting at the age of 6 wks. Untreated rats served as control. At the age of 8 and 16 wks, HepCL and IMCL in tibialis anterior muscle were measured by $^1$H-NMR-spectroscopy. At the age of 12 wks, body fat content was determined by $^1$H-NMR-spectroscopy. At the age of 18 wks, animals were killed after an overnight fast. LCACoA-concentrations in liver and longissimus dorsi muscle were analysed by HPLC. Metabolic serum parameters were determined throughout the study.
**Results:** Both treatments prevented the increase of blood glucose. MET had no effect on serum lipid parameters, whereas RGZ showed a significant lipid-lowering effect. At the age of 8 and 16 wks, IMCL in the RGZ group was significantly reduced. HepCL-levels of the rats that were treated with RGZ were not reduced at the age of 8 wks, but at the age of 16 wks. MET had only marginal reducing effects on IMCL and did not decrease HepCL. Treatment with RGZ resulted in an increase of body weight and body fat content compared with control animals, whereas MET had no effect on these parameters. Both treatments resulted in a significant decrease of muscle C16:0-CoA and C18:2-CoA, whereas C20:4-CoA was increased compared to the control group. In the liver, C16:1-CoA levels were significantly increased in RGZ-treated animals. Hepatic C18:2-CoA-levels were reduced in both treatment-groups. Treatment of obese ZDF-rats had no effect on total LCACoA-levels.
**Conclusion:** Both treatments prevented the onset of diabetes. MET had no effect on lipid parameters, neither on serum triglycerides and free fatty acids nor on HepCL and caused only a marginal reduction of IMCL. In contrast, RGZ significantly lowered both, serum and tissue lipids. As IMCL serves as surrogate for insulin sensitivity, we conclude, that RGZ, not MET, ameliorates peripheral insulin sensitivity in ZDF rats. The increased body fat content of RGZ-treated animals may be result of a PPARγ-mediated increase of adipocytes differentiation. Whether the modifications of the LCACoA-pattern are caused by modifications of desaturases activity and whether these modifications are causative in preventing the onset of diabetes remains to be investigated.

## 656

**Human recombinant soluble CD14 (sCD14) ameliorates insulin sensitivity of insulin-resistant obese mice**

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**Background and aims:** Insulin resistance in major target tissues is thought to be associated with chronic inflammation. The endotoxin LPS (lipopolysaccharide) is a potent inducer of inflammatory response. CD14 is a myeloid differentiation antigen expressed on the surface of cells of the monocyte/macrophage lineage that serves as a receptor for lipopolysaccharide (LPS) and other bacterial wall-derived components. Some obese individuals prone to develop type 2 diabetes showed decreased levels of circulating sCD14 in a recent study. We hypothesized that sCD14 could play a role in insulin resistance modulation. Therefore, the aim of this study was to investigate the effect of systemic administration of human recombinant soluble CD14 (sCD14) on insulin sensitivity.
**Materials and methods:** We evaluated blood glucose and plasma insulin concentrations in *ob/ob* mice (leptin knock-out mice) and HF mice (Mice fed with a high fat diet) treated with sCD14 and subjected to an intraperitoneal glucose tolerance test (IPGTT).
**Results:** The treatment with sCD14 did not produce significant changes, between treated and controls, in body weight, the weight of soleus muscles, the weight of epididimal adipose tissue or the ingestion of food. Fasting blood glucose (242 ± 30 mg/dl in control vs 153 ± 14 mg/dl in sCD14 treated mice, $P = 0.02$) and glucose tolerance ($P < 0.002$ by Two-way ANOVA) were significantly improved by sCD14 treatment in *ob/ob* mice. The concentration of serum insulin also decreased concomitantly with the decrease of serum glucose in mice treated with sCD14 ($P < 0.0001$ by Two-way ANOVA). HF mice fasting blood glucose levels were similar between sCD14 treated and control mice. After IP glucose loading, glycemia failed to return to the fasting baseline level after 180 min, indicating moderate glucose intolerance in HF mice. However, glucose tolerance was significantly improved by sCD14 treatment (Glucose curves were different by two-way ANOVA, $P < 0.002$). The concentrations of serum insulin increased simultaneously with the decrease of blood glucose in mice treated with sCD14.
**Conclusion:** These results show that sCD14 has a positive effect in normalizing blood glucose levels in insulin-resistant obese mice through changes in insulin action or production.

## 657

**Peripherally produced GLP-1 causes satiety**

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**Background and aims:** The truncated amidated form of glucagon-like peptide-1 (GLP-1) is a protein product of the pro-glucagon gene and secreted from ileal L-cells, pancreatic A-cells and from nerve terminals in the CNS. In the periphery, GLP-1 is secreted in response to ingested nutrients and acts to ameliorate fuel excursion associated with ingested food (e. g., by stimulating insulin release and action and by inhibiting upper gastrointesinal motility). In the CNS, GLP-1 participates in the CNS regulation of nutrient balance, since GLP-1 injected into the third cerebral ventricle of rats and mice has profound inhibitory effects on food intake and upper gastrointestinal motility. Here we studied whether endogeneously produced GLP-1 regulates ingestive behavior and gastrointestinal motility.
**Materials and methods:** Increased GLP-1 secretion was reached by intragastric infusion of sucrose with and without acarbose. Acarbose inhibits sucrose absorption by the luminal brush border enzyme α-glucosidase, and causes dumping of sucrose into the lower intestine, and as a consequence augments GLP-1 production. GLP-1, insulin and glucose levels in the blood were measured after intragastric infusion of sucrose with or without acarbose. In different sets of experiments, the effects of sucrose with or without acarbose on gastric contractions and aversive side effects were assessed. Monoclonal antibody raised against GLP-1 was used to test for specificity of effects.
**Results:** Plasma GLP-1 levels after sucrose+acarbose were significantly higher than after sucrose injection alone at t = 12, 15 and 20 min. While sucrose alone had little effect on food intake, addition of acarbose strongly augmented sucrose's anorexigenic efficacy. This effect was partly blocked by i.v. injection of antibody raised against GLP-1. The effect of acarbose+sucrose on gastric contractions did not differ from sucrose alone, but were both lower than after demi or acarbose alone. Interestingly, the effects of sucrose+acarbose on stomach contractions were not blocked by GLP-1 immunoneutralization. Rats did not develop a taste aversion in response to sucrose+acarbose, but showed a taste preference instead.
**Conclusion:** Peripherally produced GLP-1 is involved in satiety but not in the "ileal brake" mechanism. The satiating effect of peripherally produced GLP-1 is not due to aversive side effects.

## 658

**Action of GLP-1 upon glucose transport in myocytes from morbidly obese patients**

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**Background and aims:** Patients suffering of morbid obesity frequently show glucose intolerance and insulin resistance. In rat and human skeletal muscle, GLP-1 stimulates glycogen synthase *a* activity and glycogen synthesis, and this peptide, and its structurally related exendin-4 (Ex-4) and the truncated form 9–39 amide (Ex-9) also exert an increasing effect on glucose uptake, although in a lower magnitude than that by insulin. In this work we have studied the effect of GLP-1, and that of Ex-4 and Ex-9 upon glucose transport (GT), in myocytes from morbidly obese patients.
**Materials and methods:** Myotubes were established from satellite cells of dissociated *vastus lateralis* from 6 obese patients (6F; age: 44 ± 5 yr; fasting plasma glucose: 119 ± 15 mg/dl; BMI: 51 ± 2 Kg/m$^2$; HDL: 44 ± 1 mg/dl) previous informed consent given, undergoing surgery, and fused in alpha-MEM, at 5.5 mM D-glucose. For GT – measured as 2-deoxy-D-[1,2-$^3$H] glucose incorporation into the cells –, preincubation was carried out during 30 min at 37°C in a modified KRB containing 20 mM Hepes, and in the absence (control) and presence of $10^{-8}$ M each peptide; that was followed by a 5-min incubation with 2-deoxy-D-[1,2-$^3$H], at 8.6 nM D-glucose.
**Results:** In myocytes from obese patients, compared to a previously studied group of normal subjects, GT control value was lower (7.0 ± 0.5 fmol/2x10$^4$ cells, n=6, $p$=0.001 *vs* normal); yet, not only the magnitude of the respective incremental effect of insulin (90 ± 9% Δ, of control, $p$<0.0001) and Ex-4 (37 ± 5% Δ, $p$=0.002) was maintained, but also that of GLP-1 (97 ± 8% Δ, $p$<0.0001) and Ex-9 (97 ± 9% Δ, $p$<0.0001) which, in addition were even much higher, (both $p$<0.0001 *vs* normal). Despite this apparently normal sensitivity of the obese cells in response to insulin and Ex-4, and higher to GLP-1 and Ex-9, the net glucose uptake reached by Ex-4 was lower (9.6 ± 0.4 fmol/2x10$^4$ cells, $p$<0.001 *vs* normal), and so it was that by insulin (13.3 ± 0.6, $p$<0.001) whose value was undistinguishable from that by GLP-1 (14.0 ± 0.6) or Ex-9 (13.8 ± 0.6), the net GT by either of the last two peptides being also diminished ($p$<0.001) respect those found in normal cells.
**Conclusion:** In myocytes from morbidly obese patients, GLP-1, Ex-9 and Ex-4 are able, like insulin, to stimulate glucose transport despite the lower than normal basal activity of the cells. This adds support to the beneficial value of GLP-1, and at least Ex-9, in the control of the possible deleterious glucose uptake by the muscle in morbid obesity.

## 659

### Chronic treatment of obese hyperglycaemic *ob/ob* mice with exendin(9–39)amide suggests a minor role for endogenous GLP-1 in metabolic abnormalities of obesity-diabetes

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**Background and aims:** Glucagon-like peptide-1 (GLP-1) is a potent insulinotropic hormone that has been proposed to play a role in the pathophysiology of type 2 diabetes. It currently has a high profile as a potential antidiabetic agent. GLP-1 lowers blood glucose by stimulating insulin release from pancreatic beta cells, but it may also act at peripheral sites to promote glucose disposal. This study has employed the GLP-1 receptor antagonist, exendin-4(9–39)amide (Ex(9–39)) to evaluate the role of endogenous GLP-1 in genetic obesity-diabetes and related metabolic abnormalities using *ob/ob* mice.
**Materials and methods:** Acute *in vivo* antagonistic potency of Ex(9–39) was determined by i.p. injection of GLP-1 in combination with glucose (18 mmol/kg) and either equimolar GLP-1 or Ex(9–39) (25 nmol/kg plus 25 nmol/kg body weight) in obese diabetic *(ob/ob)* mice (n=8; 14–18 weeks). Plasma glucose and insulin concentrations were measured, immediately prior to (t=0 min) as well as 15, 30 and 60 min following injection. In longer-term studies, *ob/ob* mice (n=8) were chronically administered (11 days) once-daily (25 nmol/kg body weight) with Ex(9–39) or saline (9 g/l) and effects on body-weight, food intake, glucose-tolerance, insulin-sensitivity, glycated $HbA_{1c}$ and effects on feeding were measured. These measurements were repeated following a 9 day recovery period without Ex(9–39) administration.
**Results:** Area under the curve (AUC) values following injection of GLP-1 with glucose in acute studies were significantly different compared to injection of GLP-1 in combination with Ex(9–39). Potent antagonism of GLP-1 by Ex(9–39) was evident with AUC for glucose being significantly increased from 1361 ± 121 to 1914 ± 176 mmol/l.min (P<0.01) and AUC for insulin significantly decreased from 708 ± 64 to 473 ± 46 ng/ml.min (P<0.01). In longer-term studies, the bodyweight (72.7 ± 2.6g), food intake (10.5 ± 0.4g/day), insulin (53.6 ± 5.0 ng/ml) and glycated $HbA1_c$ (6.25 ± 0.5%) of *ob/ob* Ex(9–39)-treated mice did not significantly differ from *ob/ob* mice treated daily with saline (76.0 ± 2.5g, 10.3 ± 0.5g/day, 43.6 ng/ml and 5.75 ± 0.3%, respectively). These parameters did not change during or after the 9 days of treatment withdrawal. Longer-term treatment with Ex(9–39) caused minor impairment of glucose homeostasis (plasma glucose 22.2 ± 1.1 mmol/l, P<0.05) and glucose-tolerance (AUC, 1067.0 ± 36.0 mmol/l.min, P<0.05) compared to *ob/ob* mice treated with saline (17.3 ± 2.0 mmol/l and AUC, 925.7 ± 49.9 mmol/l.min, respectively). Following the 9 day period of treatment withdrawal normal glucose homeostasis (glucose 17.7 ± 1.6 mmol/l) and glucose-tolerance (AUC, 986.9 ± 142.4 mmol/l.min) were restored. The impairments in glucose metabolism observed with Ex(9–39) treatment were not associated with any impairment of insulin secretion or insulin-sensitivity.
**Conclusion:** These findings suggest that endogenous GLP-1 may play a relatively minor role in metabolic abnormalities associated with obesity-diabetes. In addition, the data suggest that involvement of GLP-1 in glucose intolerance of this animal model of type 2 diabetes may be confined to extrapancreatic glucose-lowering actions.

## 660

### Effect of 28-day treatment with exenatide (synthetic exendin-4) or sibutramine on food intake and body weight in high fat-fed rats

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**Background and aims:** In addition to its antidiabetic actions, exenatide administration for 28 days to high fat-fed (HF-Fed) mice has been reported to reduce food intake and produce a sustained reduction in body weight gain. To extend these findings, the present investigation 1) compares the effects of 28-day exenatide treatment to the marketed anti-obesity agent, sibutramine, in HF-Fed rats, and 2) examines the dose-dependent actions of exenatide in this model.
**Materials and methods:** In Experiment 1, HF-Fed male rats (58%kcal from fat; fattened for 10wks) were infused for 28 days with exenatide (30 µg/kg/day) or sibutramine (3 mg/kg/day) via subcutaneously implanted osmotic pumps (n=10–12/group, pretreatment body weight = 529±5g [mean±SEM]). In Experiment 2, HF-Fed male rats (fattened for 4 wks) were treated with 3, 10, or 30 µg/kg/day of exenatide (n=6/group, pretreatment body weight = 454±4g). For both studies, food intake and body weight were measured weekly and plasma exenatide concentrations determined on day 28.
**Results:** Treatment with exenatide or sibutramine resulted in significant body weight loss compared to HF-Fed controls throughout the 28-day treatment period (*P<0.05 *vs.* vehicle); at the single doses tested, the body weight loss was significantly greater in the exenatide *vs.* sibutramine treated animals (P<0.05 *vs.* sibutramine). The action of exenatide was dose-dependent, with significant body weight loss maintained at 28 days at the lowest dose tested (percent body weight loss = mean vehicle body weight gain -drug body weight gain/mean baseline weight*100).

| Treatment | % Body Weight Loss (cumulative) | | | |
|---|---|---|---|---|
| | *Day 7* | *Day 14* | *Day 21* | *Day 28* |
| ***Experiment*** 1 | | | | |
| Exenatide 30 µg/kg/day | 11.5* ± 0.8 | 10.7* ± 1.2 | 10.1*± 0.7 | 9.6* ± 0.6 |
| Sibutramine 3 mg/kg/day | 6.1* ± 0.5 | 6.4* ± 0.8 | 6.0* ± 0.5 | 5.4* ± 0.6 |
| ***Experiment 2*** | | | | |
| Exenatide 3 µg/kg/day | 9.6* ± 2.1 | 8.4* ± 2.3 | 6.3* ± 2.0 | 4.3* ± 2.2 |
| 10 µg/kg/day | 12.1* ± 0.7 | 16.4* ± 1.5 | 10.8* ± 0.8 | 15.1* ± 1.5 |
| 30 µg/kg/day | 10.0* ± 1.0 | 12.9* ± 1.3 | 8.4* ± 1.2 | 11.7* ± 1.2 |

In parallel, food intake was reduced during weeks 1 and 2 in exenatide- and sibutramine-treated rats compared to HF-Fed controls; during week 4, all groups displayed equivalent food intake. Nonlinear regression analysis derived a plasma concentration-response relationship for body weight with an $EC_{50}$ of 14.3pM±0.004 log units.
**Conclusion:** These results demonstrate an effect of exenatide on body weight in chronically exposed rats fed a high fat diet.

## 661

**NO-1886, a lipoprotein lipase activator, improves obesity by accelerating uncoupling protein 3 in rats**

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**Background and aims:** Although NO-1886 shows anti-obesity effects in high-fat induced obesity in rats, the mechanism is unclear. To clarify the mechanism, we studied the effects of NO-1886 on the expression of uncoupling protein (UCP) 1, UCP2, and UCP3 in rats.
**Materials and methods:** NO-1886 was mixed with a high-fat chow to supply a dose of 100 mg/kg to 8 month old male SD rats. The animals were fed the high-fat chow for 8 weeks. At the end of the administration period, the brown adipose tissue (BAT), mesenteric white fat, and soleus muscle were collected and levels of UCP1, UCP2, and UCP3 mRNA were determined by northern blotting.
**Results:** NO-1886 suppressed the body weight increase after the 8 week administration (650 ± 43 vs 730 ± 73g, P<0.05). NO-1886 also suppressed fat accumulation in visceral (52.27 ± 11.6 vs 81.9 ± 16.1g, P<0.01) and subcutaneous (47.8 ± 20.1g vs 76.6 ± 20.9g, P<0.05) tissues. NO-1886 increased the levels of plasma total cholesterol and high-density lipoprotein cholesterol. In contrast, this compound decreased the levels of plasma triglycerides, glucose and insulin. NO-1886 increased LPL activity in soleus skeletal muscle (0.068 ± 0.013 vs 0.051 ± 0.013 μ moles FFA/min/g tissue, P<0.05), without affecting levels in the mesenteric white adipose tissue and BAT. NO-1886 increased the expression of UCP3 mRNA in soleus 2.73 fold (P<0.01) and increased the expression of UCP3 mRNA in mesenteric adipose tissue 1.87 fold (P<0.05) compared to control group without affecting the levels of UCP3 in BAT. In addition, NO-1886 did not affect the expression of UCP1 and UCP2 in BAT, mesenteric white adipose tissue and soleus skeletal muscle.
**Conclusion:** NO-1886 increased the expression of UCP3 mRNA but did not affect the expressions of UCP1 and UCP2 mRNAs. NO-1886 increased LPL activity in soleus skeletal muscle without increasing LPL activity in adipose tissue. These results indicate that NO-1886 improves high-fat feeding obesity in rats by increasing LPL activity in skeletal muscle, resulting in increased UCP3 expression. The mechanism of NO-1886's anti-obesity effects may be the enhancement of LPL activity in skeletal muscle, and the accompanying increase in UCP3 expression.

## 662

**Reduction of adiposity in C57BL/6J mice by dietary omega-3 polyunsaturated fatty acids of marine origin.**

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**Background and aims:** Marine fish oils with a high content of omega-3 polyunsaturated fatty acids (n-3 PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) acid improve various metabolic disorders associated with obesity and *type 2* diabetes. Fish oils exert their beneficial effects by triggering complex changes in gene expression, fatty acid oxidation and plasma membrane composition in a number of target organs including adipose tissue. Our aim was to test several n-3 PUFA diets of well-defined composition with respect to their effects on body weight, adiposity and plasma concentration of various metabolites.
**Materials and methods:** Groups ($n$ = 7) of 3-mo-old male C57BL/6J mice weaned onto a standard chow diet [fat ~ 4% (w/w)] were randomly assigned to one of several semisynthetic, high-fat [20% (w/w) fat] diets, where 44% (w/w) of the fat component was replaced by n-3 PUFA concentrates (EPAXproducts, Pronova Biocare, Norway) as follows: 1) lard (palmitic and oleic acid; diet "L"), 2) lard + EPAX 2050 TG (22% EPA, 42% DHA; "L+D"), 3) flax-seed oil (plant omega-3 PUFA; "Ln"), 4) flax-seed + EPAX 2050 TG ("Ln+D"), 5) Corn oil (plant omega-6 PUFA; "K"), 6) Corn oil + EPAX 4510 TG (50% EPA, 10% DHA; "K+E"), and 7) Corn oil + EPAX 1050 TG (10% EPA, 50% DHA; "K+D"). Moreover, a combination of flax-seed oil and EPAX 2050 TG was also tested, where the percentage of n-3 PUFA concentrate reached only ~ 15% of a total fat content (diet "Ln+Dlow"). At the end of a feeding experiment (1 to 2 mo), subcutaneous and gonadal fat depots were dissected, weighed and tissue cellularity was analyzed by fluorometric quantitation of DNA and gene expression by real time RT-PCR. Plasma concentrations of various metabolites [triacylglycerol (TG), FFA] and leptin (RIA kit) were also measured.
**Results:** Body weight of mice treated with n-3 PUFA diets Ln+D, K+D and K+E were significantly lower than in their counterparts fed Ln or K diet alone. In mice fed n-3 PUFA, plasma TG were relatively low, averaging ~ 30% of the levels found in the control groups. The TG-lowering effect of L+D diet was seen already after 1 mo of feeding. Plasma FFA were depressed only in K+D group. Circulating leptin was decreased by 60% after 2 mo on the diet in Ln+D and K+D groups, while in the K+E group leptin declined by only ~ 40%. Accordingly, the weight of gonadal fat was depressed more in Ln+D and K+D than K+E diet group (Ln, 357.2 ± 56.6 vs. Ln+D, 182.0 ± 13.1 mg, $P$ < 0.01; K, 721.7 ± 131.7 mg vs. K+D, 355.4 ± 38.6 mg vs. K+E, 449.7 ± 79.0 mg, $P$ < 0.05 for K vs. K+D). The effect on adiposity could be likely attributed to changes in adipocyte size, since n-3 PUFA diets increased DNA concentration by 30–50%. The weight of subcutaneous fat was less affected by the n-3 PUFA diets tested. Ln+D diet increased expression of *Glut4* by ~ 35% and ~ 70% in subcutaneous and gonadal fat, respectively, while *leptin* expression was reduced by ~ 75% in both fat depots. It is noteworthy that Ln+Dlow diet, in which n-3 PUFA concentrate constituted only ~ 15% of a total fat content, was ineffective with respect to all parameters measured except for plasma TG and gene expression in fat depots.
**Conclusion:** Our results show fat depot-specific and dose-dependent effects of dietary n-3 PUFA. Higher content of DHA seems to confer stronger effect irrespective of a "fat background" in the diet, thus offering a potential strategy for the treatment of obesity and metabolic syndrome.

## 663

**Chronic administration of the Kir 6.2/SUR 1 selective $K_{ATP}$ channel opener, NN414, does not affect Body Weight in 1 year old male Wistar rats**

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**Background and aims:** NN414 is a Kir6.2/SUR1 selective $K_{ATP}$ channel opener that improves oral glucose tolerance in normal and Zucker obese rats as well as ameliorates the progression of diabetes in Psammomys Obesus through the induction of β-cell rest.
Obese, glucose intolerant subjects experience postprandial hyperinsulinemia which could contribute to hyperphagia. Hypothetically, inhibition of postprandial hyperinsulinemia by acute β-cell rest should reduce food intake and consequently body weight. The aim of the present study was to assess the effects of chronic NN414 on body weight in 1 year old male Wistar rats.
**Materials and methods:** Male Wistar rats were kept on a reverse dark: light cycle (lights off at 9 am) and were given free access to tapwater and Purina 5008 chow. Prior to NN414 treatment, the rats were allocated to groups with matching oral glucose tolerance. For three weeks, NN414 was administered once daily by gavage immediately before the onset of the dark phase/ eating. The four groups (n=7) received either NN414 (1 mg/kg (A), 3 mg/kg (B), 10 mg/kg (C)) or vehicle (D). Following the first and the last dose, the acute effects on blood glucose, plasma insulin and food intake were registered for 4 hours. $HbA_{1C}$ was assessed before and after the treatment period. Body weight was followed throughout the study. Data are mean ±SEM and analysed by one way ANOVA.
**Results:** The acute response to NN414 was maintained throughout the study. NN414 (last dose) induced transient hypoinsulinemia (4 h mean: 706 ± 175 pM (A); 719 ± 47 pM (B); 152 ± 9 pM* (C) and 728 ± 102 pM (D);P<0.001) accompanied by hyperglycemia (4 h mean: 6.3 ± 0.2 mM (A); 8.2 ± 0.6 mM* (B); 12.9 ± 0.5 mM** (C) and 6.1 ± 0.1 mM (D); P<0.0001) without affecting food intake (P=0.46). Changes in $HbA_{1C}$ (−0.06 ± 0.16% (A); 0.36 ± 0.08% (B); 0.29 ± 0.17% (C); 0.11 ± 0.07% (D); P=0.74) and body weight (−0.9 ± 2.8 g (A); 1.1 ± 2.8 g (B); 1.2 ± 2.8 g (C) and −4.8 ± 3.1 g (D); P=0.43) were not significantly different.
**Conclusion:** Chronic administration of the Kir 6.2/SUR1selective $K_{ATP}$ channel opener NN414 induced transcient hypoinsulinemia without affecting foodintake or body weight in 1 year old male Wistar rats. Furthermore, the daily episodes of transcient hyperglycemia did not affect $HbA_{1C}$.

## 664

**Intranasal insulin reduces body fat in healthy men but not in women**

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**Background and aims:** Insulin acts in the central nervous system to reduce food intake and body weight and is considered a major adiposity signal. After intranasal administration in humans, insulin enters the cerebrospinal fluid compartment and alters brain functions in the absence of substantial absorption into the blood stream. The aim of this study was to determine whether prolonged treatment with intranasal insulin reduces body fat in humans.
**Materials and methods:** Following a baseline period of 2 weeks, 40 healthy human subjects were intranasally administered with regular human insulin (4 × 40 IU/day) or placebo (12 men and 8 women in each group) over a period of 8 weeks in a double blind manner. Body weight, body composition, measures of sympathetic activity, resting energy expenditure, ratings of feelings of hunger, and hormonal parameters were assessed every week during the study.A follow-up examination of the male subjects took place 4–5 months thereafter.
**Results:** Blood glucose and plasma insulin levels were identical in both treatment conditions. The insulin-treated men lost 1.28 ± 0.71 kg of body weight and 1.38 ± 0.59 kg of body fat, and their waist circumference decreased by 1.63 ± 1.17 cm (each $P<.05$ as compared to the placebo condition with the baseline values serving as covariate). Plasma leptin levels dropped on average by 27% during treatment ($P<.05$). Hunger ratings after 8 weeks of treatment decreased during the final examination ($P<.02$). Comparisons concerning all other parameters, including resting energy expenditure and heart rate variability (expressed as the ratio of low to high frequency power), failed to reveal significant effects of long-term insulin administration. Contrasting with these effects, the insulin-treated women did not lose body fat, but gained 1.04 ± 0.38 kg of body weight due to a rise in extracellular water ($P<.02$). In the follow-up examination of the men conducted 17.6 ± 0.52 weeks after cessation of treatment, the differences between the insulin and placebo groups had vanished and body fat as well as body weight were comparable.
**Conclusion:** Our results provide for the first time in humans a direct confirmation of the concept that insulin acts as a negative feedback signal to the brain in the regulation of adiposity, and point to a differential sensitivity to the catabolic effects of insulin in both sexes. Considering the impaired blood-to-brain transport of insulin associated with obesity, intranasal insulin could be a promising tool to promote weight loss in obese men.

Effects of 8 weeks of intranasal insulin administration in men. Means are baseline adjusted.

| | Insulin | Placebo | p< |
|---|---|---|---|
| Body weight (kg) | 78.12 ± 0.57 | 79.84 ± 0.57 | 0.05 |
| BMI (kg/m²) | 23.02 ± 0.17 | 23.51 ± 0.17 | 0.05 |
| Body fat (kg) | 12.25 ± 0.61 | 14.12 ± 0.61 | 0.05 |
| Waist circumference (cm) | 81.06 ± 0.84 | 83.43 ± 0.84 | 0.05 |
| Leptin (ng/ml) | 2.06 ± 0.29 | 2.86 ± 0.25 | 0.05 |
| Hunger gradient | −0.36 ± 0.36 | 0.99 ± 0.36 | 0.02 |

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# PS 53
# Nutrition

## 665

**Dietary fat content modifies liver fat in obese women**

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**Background and aim:** Fat accumulation in the liver has been shown to be closely correlated with hepatic insulin resistance, even independent of body weight. The reason for interindividual variation in liver fat content is unknown. Cross-sectional data suggest dietary fat content may influence liver fat but this possibility has not been directly tested in humans. The aim of the present study was to compare effects of a low and a high fat diet on liver fat and in obese women.
**Materials and methods:** Ten non-diabetic obese women (age 43 ± 2 yrs (mean±SEM), BMI 33 ± 1 kg/m²) were placed in a randomized cross-sectional fashion on 2 2-week isocaloric diets containing either 16% (low fat diet) or 56% (high fat diet) of total energy as fat. Liver fat (proton spectroscopy), intra-abdominal and subcutaneous fat (MRI) and rates of glucose and lipid oxidation (indirect calorimetry) were determined before and at the end of each diet.
**Results:** There were no changes in body weight or amounts of intra-abdominal and subcutaneous fat after the diets. Liver fat at baseline averaged 10 ± 2% and decreased by 20 ± 9% during the low fat diet and increased by 35 ± 21% during the high fat diet (p=0.014 for liver fat after low vs. high fat diets, p=0.042 for change in liver fat by the low vs. high fat diet). Fasting serum insulin averaged 70 ± 11 pmol/l at baseline and decreased to 60 ± 8 pmol/l during the low fat diet (p=0.007 vs. before low fat diet) and increased to 81 ± 15 pmol/l during the high fat diet (p=0.040 vs. before high fat diet, p=0.005 for change in serum insulin during low vs. high fat diet). Although fasting serum adiponectin did not change significantly during the diets, the change in serum adiponectin during the low fat diet correlated inversely with the change in liver fat during the low fat diet (r =−0.78, p<0.01). After the high fat diet, a low rate of lipid oxidation was significantly correlated with high liver fat (r =−0.76, p=0.011).
**Conclusion:** These data show that lowering of dietary fat content decreases liver fat in humans.

## 666

**Glycaemic control in Type 2 diabetes tube-fed patients after brain damage during long-term treatment with a new low carbohydrate, high monounsaturated fatty acid containing enteral formula versus a standard-like formula. A randomised, prospective, controlled, double-blind, multi-centre trial**

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**Background and aims:** Metabolic control in diabetic patients who require tube feeding after brain damage can be frequently complicated due to intercurrent infections with elevated levels of "stress hormones" and increased insulin requirements, lack of physical activity and nutritional problems. A short-term pilot study showed that a specific enteral formula can help to improve glycaemic control in diabetic patients. The aim of this study was to assess the effect of a long-term treatment with a new low carbohydrate, high monounsaturated fatty acid containing enteral formula (Diben, Fresenius Kabi) in comparison with a standard-like enteral formula on daily insulin requirement and glycaemic control in tube-fed type 2 diabetic patients after brain damage of various origins.
**Materials and methods:** The study was designed as a randomised, prospective, controlled, double-blind, multi-centre, parallel group study, planned as two-stage adaptive procedure. Insulin treated type 2 diabetic patients with $HbA_{1C} \geq 7.0\%$ and/or fasting blood glucose > 120 mg/dl who required long-term enteral tube feeding due to neurological disorders were randomised to receive 27 kcal/kg BW (max. 2025 kcal/d) of either Diben (test group) or

an isoenergetic, isonitrogenous enteral formula (control group) for up to 84 days. Besides other variables, fasting blood glucose levels and insulin dosages were assessed daily, $HbA_{1C}$ was measured on days 0, 28, 56, and 84.
**Results:** 78 patients (39 in each group) were included in stage I of the study. Median values of age and BMI were 71 and 25.6 (test group) vs. 72 and 26.5 (control group). Median values of baseline and of changes from baseline after 12 weeks were as follows (test group vs. control group): total daily insulin requirement (U) baseline 38.7 vs. 44.0, changes from baseline −6.0 vs. 0.0 (p=0.0024); fasting blood glucose (mg/dl) baseline 143.0 vs. 158.0, changes from baseline −28.6 vs. −1.4 (p=0.0068); $HbA_{1C}$ (%) baseline 7.7 vs. 7.4, changes from baseline −0.8 vs. 0.0 (p=0.0016). No severe hypoglycaemia was reported during study treatment. Both formulas were well tolerated.
**Conclusion:** Treatment with the new enteral formula significantly reduced insulin dosages, fasting blood glucose and $HbA_{1C}$ during long-term enteral nutrition compared to treatment with the standard enteral formula. In spite of a lower insulin requirement the new enteral formula provided better diabetes control than the standard enteral formula.

## 667

**Dietary omega-3 polyunsaturated fatty acids decreases insulin resistance and markers of oxidative stress in patients with diabetic peripheral neuropathy and obesity**

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**Background and aims:** The aim of this study was to assess the effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on the insulin resistance parameters, the activities of $Na^+$, $K^+$-ATPase and $Ca^{2+}$, $Mg^{2+}$-ATPase in the membranes of RBCs' and the levels of the $^{125}$I-6-keto-prostaglandin $F_{1\alpha}$ (6-ketoPGF$_{1\alpha}$) and $^{125}$I-thromboxane $B_2$ ($TXB_2$) in the blood plasma, activities of superoxide dismutase (SOD), gluthation peroxidase (GPO) in the RBCs', lipid peroxidation of RBCs', concentration of conjugated dienes (CD), platelet aggregation in Type 2 diabetic patients with peripheral neuropathy (DPN) and obesity.
**Materials and methods:** 37 patients (56 ± 7 years, 24 m/13f), BMI (36,5 ± 4,78 kg/m$^2$) were allocated into two treatment groups. The 1st group (n=21) was receiving capsules of fish oil every day (2,0 g EPA, 2,0 g DHA and 0,1% α-tocopherol acetate) 2 months and the 2nd group (n=16) was receiving placebo capsules of olive oil. All patients were on the same diet. Fasting serum glucose and insulin levels were determined and the homeostasis assesment model (HOMA) index was calculated (fasting serum glucose × insulin level/22,5). ADP-induced platelet aggregation was measured by automatic system. Parameters of platelet function, other biochemical investigations were observed at baseline state and at the end of 1 and 2 months period. Statistics: one way analysis of variance (ANOVA).
**Results:** It is assumed that patients showed increase of insulin resistance and oxidative stress parameters. Besides, there was a decrease of the activities of $Na^+$, $K^+$-ATPase, $Ca^{2+}$,$Mg^{2+}$-ATPase, SOD and GPO in the RBC's membranes. There is a considerable increase in the $TXB_2$ level (246,3 ± 16,3 pg/ml, p<0,001), erythrocyte lipid peroxidation, level of CD and a decrease of 6-keto-PGF$_{1\alpha}$ in serum. Analysis of aggregatory curves shows that platelets in patients with DPN and obesity began to aggregate earlier and the speed (0,77 ± 0,02 U/min, p<0,001) and the stage of aggregation (32,64 ± 1,26 mU/min, p<0,01) increases. After 2 months of treatment there were revealed positive changes of the HOMA index parameters (p <0,01), oxidative stress parameters, a degree and speed of an aggregate of thrombocytes was marked; decrease in $TXB_2$ level (164,3 ± 16,2 pg/ml, p<0,001), level of CD (p<0,01) with simultaneous increases in activities of $Na^+$, $K^+$-ATPase (from 0,04 ± 0,003 to 0,09 ± 0,004 mMol $P^3$/mg protein per 1 hour, p<0, 001), $Ca^{2+}$, $Mg^{2+}$ -ATPase and GPO (p<0,001) and the concentration of 6-ketoPGF$_{1\alpha}$ in the first group. Increasing the activities of membrane-bound enzymes lead to increasing of RBC's deformability. This effect is conditioned also by increased prostacyclin $I_2$ production as well as by inhibition of platelet activity. The above-stated cganges were accompanied by positive dynamics of tool and functional samples permitting to troubleshoot a DPN in patients. Also, we observed significant improvement of cardiovascular autonomic tests, HRV parameters, decreasing of QTc interval (p<0,01). Therefore it seems that a 4,0 g fish oil treatment during following 2 months result the tendency of normalizing the state of prostacyclin $I^2$-thromboxane $A_2$ system, activity of membrane-bound enzymes.
**Conclusions:** EPA and DHA at moderate doses decreases insulin resistance, improving of platelet aggregattion parameters and antioxidant status of Type 2 patients with DPN and obesity and may be used for the treatment of these patients.

## 668

**The effects of hypocaloric and hypolipidic diet on metabolic syndrome patients**

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**Background and aims:** The diagnosis of metabolic syndrome can be made when 3 or more of the 5 risk determinants (waist circumference, blood pressure, HDL-cholesterol, triglycerides and fasting glucose) are present. This study was designed to measure the impact of an educational program comprising of diet therapy and physical exercises, on metabolic syndrome patients.
**Materials and methods:** A number of 68 patients – 29 men (42.35%) and 39 women (57.65%), age 47.6 ± 8.6 years, obese (BMI>25 kg/m$^2$), with hypercholesterolemia (total cholesterol>220 mg/ml, HDL-cholesterol<40 mg/ml), type 2 diabetes mellitus (glycemia>110 mg/dl) and hypertension (systolic BP>140 mmHg and diastolic BP>90 mmHg) – were included in an educational program, consisting of diet and exercises. The lifestyle changes of these patients aimed at decreasing cardiovascular risk. The educational program included a description of the risk factors and their primary prevention, a distinct diet (with decreased calory, lipid and sodium intake) and moderate intensity exercises (at least 3 days/week, for a minimum of 30 minutes). Every patient included in this program was clinically reevaluated at two months interval. Measurements of blood pressure, glycemia and lipid levels were taken after 6 months.
**Results:** An average weight loss of 5.9 ± 2.8 kg of the initial weight was recorded. Body-mass index was reduced from 29.2 ± 4.4 kg/m$^2$ to 26.1 ± 4.3 kg/m$^2$ (p<0.05). Systolic BP dropped from 150 ± 25 mmHg to 135 ± 15 mmHg (p=0.05). Diastolic BP decreased from 100 ± 15 mmHg to 85 ± 10 mmHg (p<0.05). Total cholesterol dropped from 223 ± 33 mg/dl to 185 ± 24 mg/dl (p<0.05), triglycerides from 163 ± 67 mg/dl to 129 ± 54 mg/dl (p<0.01), glycemia from 129 ± 24 mg/dl to 118 ± 18 mg/dl (p=0.05) and HDL-cholesterol increased from 34 ± 12 mg/dl to 40 ± 18 mg/dl (p<0.05) after 6 months.
**Conclusion:** The present study demonstrates the positive impact of a short educational program doubled by diet and physical exercises. A good metabolic control of metabolic syndrome patients can be attained in this way with relative ease. The results further emphasize the need for education regarding weight loss and the management of associated risk factors, together with improper diets, alcohol consumption, sedentarism, smoking and stress.

## 669

**Adolescent obesity is associated with excess consumption of sugar-free soft drinks**

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**Background and aims:** Adolescent obesity and the role in its etiology of soft drink and fast-food consumption have been the focus of concern among scientific, medical, educational, and governmental communities and a frequent topic of recent press coverage. Our series of 1298 high school students examined the association between soft-drink consumption and body mass index (BMI).
**Materials and methods:** All students from one public high school (40% non-Hispanic White, 54% Hispanic, 6% other) were invited to participate in a health screening program from the fall of 2000 through 2003. The screening included height, weight, percent body fat (Tanita® Body Composition Analyzer), fingerstick AlC (DCA2000®+), and a health behavior questionnaire. This report is limited to 1207 non-Hispanic White and Hispanic students who responded to a question regarding soft-drink consumption.
**Results:** We found 21.6% of non-Hispanic White and 40.9% of Hispanic participants drank 2 or more soft drinks per day, and 8.0% of non-Hispanic White and 14.2% of Hispanic participants drank 3 or more soft drinks per day. 147 (12.2%) had a BMI greater than the 95th percentile for age and sex. BMI exceeded the 95th percentile in 17.2% of those who drank 2 or more soft drinks per day, and in only 9.7% of those who drank fewer than 2 soft drinks per day ($\chi^2$=13.92, df=l, p <0.001). Consumption of 2 or more soft drinks per day was associated with a higher BMI z-score (zBMI) (F=4.25, p=0.039, controlled for sex and ethnicity). Interestingly, the association was very strong for 2 or more *sugar-free* soft drinks per day (F=8.79, p=0.003) but not for 2 or more regular soft drinks (F=0.22, p=0.636). Among non-Hispanic White students, those who drank 2 or more soft drinks (regular or

sugar-free) per day had significantly higher zBMI (F=7.02, p=0.008). This finding did not hold for Hispanic students (F=0.46, p=0.499), but higher zBMI was associated with drinking 3 or more soft drinks per day (F=5.00, p=0.026).
**Conclusion:** In this study the association between soft drink consumption and obesity was driven by the consumption of sugar-free soft drinks. Although this may be partially explained by students beginning to watch calories as they become overweight, soft drink consumption is likely part of a behavioral pattern of snacking and choosing non-nutritious foods for meals. Interventions to reduce obesity clearly need to address the overall eating patterns and foods associated with soft drink consumption, rather than the reduction in calories represented by switching to sugar-free soft drinks.
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## 670

**Long term effect of high soluble fibres supplementation on clinical features of metabolic syndrome: a randomised, double-blind clinical trial on psyllium husk vs. guar gum**
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**Background and aims:** Increasing dietary fibre intake is widely recommended as a safe and practical approach for cholesterolemia reduction and glycaemia control in diabetic subjects. Among dietary fibres, the soluble ones appear to be the most effective, however there are no direct comparison trials between them. The aim of this study is to compare the metabolic effect of high supplementation of pyllium husk and guar gum on metabolic syndrome affected patients.
**Materials and methods:** Sixty-four age- and sex matched non-smoking patients who complied to the NCEP-ATP III criteria for the metabolic syndrome diagnosis were recruited and randomised to the psyllium group or the guar group at a doses of 3.5 gr t.i.d. before meals, and followed for 6 months. At baseline, all the patients had a stabilized therapy for cardiovascular risk factors. The following parameters have been monitored: BMI, waist circumferences, TC, HDL-C, TG, basal glycaemia, systolic and diastolic blood pressure. Side effects were also constantly registered.
**Results:** While body weight decreased significantly in both group ($-1.4 \pm 0.7$ kg), no difference has been found as it regards waist circumference. The psyllium group experienced a significant reduction in systolic blood pressure ($-4.1 \pm 0.5$ mmHg), TC ($-12.2 \pm 3.4$ mg/dL), TG ($-10.9 \pm 4.7$ mg/dL), and basal glycaemia ($-6.2 \pm 2.9$ mg/dL), while the guar group only of TC ($-9.9 \pm 4.8$ mg/dL) and basal glycaemia ($-6.4 \pm 3.7$ mg/dL). Diastolic blood pressure and HDL-C plasma level were not modified by both treatment regimens ($p>0.05$). At the end of the supplementation period, 12.5% of subjects treated with psyllium and 3.12% of those treated with guar did not comply with the criteria for the metabolic syndrome diagnosis ($p<0.05$). The drop out rate was 6.25% in the psyllium group and 12.5% in the guar group ($p<0.05$), mainly because of gastrointestinal side effect.
**Conclusion:** In our patients, psyllium supplementation appeared to be more efficacious and safe in controlling component of the metabolic syndrome.
*Thanks to Nathura S.r.l., Collecchio (RE), Italy for kind furnishment of both fibres*

## 671

**An olive oil-enriched diet increases GLP-1 release and, after 36 days, augments insulin secretion and improves oral glucose tolerance in normal rats**
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**Background and aims:** Diets enriched in monounsaturated fatty acids have been shown to benefit glycemic control. The present study aims at measuring, in normal rats, the GLP-1 response to oral intake of an olive oil-enriched diet (OO), and at assessing the long-term effects of such a diet on the GLP-1 content of the gastrointestinal tract, as well as the plasma glucose and insulin pattern during an oral glucose tolerance test (OGTT).
**Materials and methods:** The plasma concentration of GLP-1 was measured before and after intake of the control and OO diet by meal-trained rats. Plasma glucose and insulin concentrations were measured during an OGTT (1.2 mg glucose/g body wt.) in rats fed for 36 days the control or OO diet. The GLP-1 content of the gastrointestinal tract was measured, in both groups of rats, at day 50.
**Results:** In the meal-trained rats, the mean increment in plasma GLP-1 concentration at min 10 and 20 was $1.39 \pm 0.23$ ng/ml higher (d.f.=6; $P<0.001$) in the rats given access to the OO diet rather than control diet. Relative to the initial value (day 0), the gain in body weight at day 50 was higher, in both male and female rats, in the animals fed the OO diet rather than the control diet. Thus, such a gain was, in the former case, $29.4 \pm 6.2\%$ higher (d.f.=10; $P<0.001$) than that found in rats of the same sex exposed to the control diet. At day 50, the GLP-1 content of the jejunum, ileum, colon and cecum was not significantly different in the two groups of rats. At day 36, however, the paired increment in plasma glucose concentration at min 15 and 30 of the OGTT represented, in the rats receiving the OO diet, only $61.6 \pm 15.1\%$ (d.f.=30; $P<0.02$) of that found in the control group. This coincided with the fact that the paired increment in plasma insulin concentration in the same samples was $116.2 \pm 46.8\%$ higher (d.f.=29; $P<0.02$) in the rats exposed to the OO diet than in the control group.
**Conclusion:** The intake of an olive oil-enriched diet, as distinct from control diet, provokes, in normal rats, a higher release of GLP-1. Long-term exposure to the OO diet also augments body weight gain, improves glucose tolerance and increases the secretory response of insulin-producing cells to oral glucose administration.

# PS 54
# Metabolic syndrome: life style

## 672

**Continuous glucose monitoring in non-diabetes obstructive sleep apnoea subjects**

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**Background and aims:** Obstructive sleep apnoea syndrome (OSAS) has been shown to be associated with insulin resistance and high prevalence of type 2 diabetes. The effect of continuous positive airway pressure (CPAP) treatment on blood glucose is unknown. The aim of our study was to assess the effect of CPAP on blood glucose assessed with the use of continuous glucose monitoring system (CGMS) in non-diabetes subjects.
**Materials and methods:** The study subjects were 7 persons with OSAS (5 male, 2 female, mean age 51.0 ± 7.0 years, mean apnoea-hypopnoea index [AHI] 47.3 ± 34.1), the controls were 7 age-matched subjects without OSAS (5 male, 2 female, mean age 48.0 ± 12.8 years, AHI 3.3 ± 2.7). OSAS subjects underwent overnight polysomnography (Erich Jaeger, Hoechberg, Germany) examinations, a diagnostic one and one with CPAP, with concomitant continuous glucose monitoring (CGMS, MiniMed, USA) on both occasions. All subjects had oral 75 g glucose tolerance test (OGTT) with fasting plasma insulin assay performed.
**Results:** Body weight of the OSAS patients was significantly greater than the controls: 103.5 ± 19.3 vs 82.6 ± 13.4 kg ($p<0.05$), BMI 35.6 ± 5.7 vs 27.6 ± 3.1 kg/m$^2$ ($p<0.01$), respectively. OGTT values were similar between the OSAS patients and controls: 0 min – 101 ± 12 and 96 ± 16 mg/dl, 60 min – 185 ± 47 and 176 ± 56 mg/dl, 120 min – 125 ± 69 and 93 ± 21 mg/dl, respectively ($p>0.05$), with 120 min values correlating with baseline AHI in the OSAS patients and controls ($r=0.61$; $p<0.05$). However, fasting plasma insulin was significantly greater in OSAS patients than in the controls: 93.5 ± 40.2 vs 28.6 ± 11.6 pM ($p<0.01$). CPAP treatment in OSAS subjects resulted in significant reduction of AHI±on average by 80% from baseline. Unexpectedly, mean values of overnight continuous glucose monitoring were significantly higher during CPAP than during the diagnostic night: 77 ± 9.8 vs 66.3 ± 7.2 mg/dl ($p<0.05$).
**Conclusion:** CPAP treatment might have an immediate effect of slightly increasing blood glucose, which could be the result of ineffective or unstable insulin action in highly insulin resitant OSAS subjects. However, this observation should be confirmed in the larger study group.

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**Appreciation of the quality of glycemic control of Type 2 diabetes in daily clinical practice**

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**Background and aims:** The prevention of degenerative complications of type 2 diabetes is based for part on the quality of glycaemic control, which can be evaluated by HbA1 c level. Nevertheless, other indicators such as fructosamine and daily glycaemic levels (8am, 11am, 2pm, 17pm) can bring complementary informations, particularly in the course of type 2 diabetes. The aim of this work is to analyze the links between these different parameters and then to deduce consequences for the supervision and the processing of type 2 diabetes.
**Materials and methods:** This study is based on a population of 880 type 2 diabetics who undertake the control of their disease during a one day hospitalization. None of these subjects receives insulin. The practiced statement includes, with the research of complications, an evaluation of the glycemic control by measurement of HbA1 c and fructosamine levels and by glycemia levels at 8am, 11am, 2pm and 5pm.
**Results:** The highest glycemia is recorded at 11am (1.80 ± 0.6 g/l), the lowest at 5 pm (1.38 ± 0.5 g/l) while 8am glycemia is 1.54 ± 0.4 g/l. The best correlations are obtained between the 11 hours glycemia and HbA1 c ($r= 0.61$ $p< 0.001$) on one hand and between the 11 hours and fructosamine ($r= 0.62$ $p< 0.001$) on the other hand.
**Conclusion:** These results underline the complementary character of HbA1 c, fructosamine and daily glycemias. 11am glycemia is very informative on glycemic control. Therapeutic modifications and informations provided during sessions of education have to take into account the 5pm glycemia which is the lowest of the day. Finally, morning glycemia, which can be immediately provided, is a good indicator of the HbA1 c level. Thus, a HbA1 c level of 7% corresponds to a 8am glycemia of 1.30 g/l.

## 674

**Metabolic syndrome in Adult-onset Latent Autoimmune Diabetes (LADA)**

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**Background and aims:** LADA belongs to autoimmune type 1 diabetes (T1DM), and is characterized by islet beta cell loss and absolute insulin deficiency. Despite the fact that insulin resistance does exist in LADA and classic T1DM patients, no studies have been reported on the relation of T1DM with metabolic syndrome (MS). The study was to investigate the association of LADA with MS.
**Materials and methods:** One hundred LADA, 869 T2DM and 68 classic T1DM patients were enrolled. And LADA patients were divided into two subgroups according to a GAD-Ab index of 0.3, that is, high titer group (LADA-type 1, n=30) and low titer group (LADA-type 2, n=70). Serum lipid and 24 hour urinary albumin excretion were measured. MS and its components were diagnosed based on the working definition proposed by WHO in 1999.
**Results:** (1) About 44.0% (44/100) of LADA, 16.2% (11/68) of classic T1DM and 54.5% (474/869) of T2DM patients complicated with MS. The prevalence of MS in LADA-type 1 and LADA-type 2 patients was 26.7% (8/30) and 51.4% (36/70) respectively. (2) The proportion of MS in LADA was higher than that in classic T1DM patients (44.0% vs 16.2%, $P<0.01$), but was not significantly different from that in T2DM (44.0% vs 54.5%, $P>0.05$). The proportion of MS in LADA-type 1 patients was lower than that in T2DM patients (26.7% vs 54.5%, $P<0.01$), but was not significantly different from that in classic T1DM (26.7% vs 16.2%, $P>0.05$). (3) The prevalence of LADA in diabetic patients with MS was 8.3%, of which approximately 85% had low GAD-Ab titers (LADA-type 2). The prevalence of LADA-type 1 in diabetic patients with MS was lower than that in patients without MS (1.5% vs 4.3%, $P<0.01$), but there was no statistical difference in prevalence of LADA / LADA-type 2 in patients with or without MS.
**Conclusion:** MS does exist in two subtypes of LADA patients, with LADA-type 2 markedly related with MS, suggesting the presence of MS could not exclude the diagnosis of LADA.

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**Prevalence of metabolic syndrome and its components in Type 2 diabetic patients of northern Italy: MEDUSA study (MEtabolic Disease Under Specific Assessment) preliminary report**

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**Background and aims:** Type 2 Diabetes is one of the major components of the metabolic syndrome, recently redefined by the National Cholesterol and Educational Program (NCEP) Expert Panel (ATP III). It is known that patients with Type 2 diabetes have an increased risk of cardiovascular complications, moreover the metabolic syndrome seems to be an independent predictor of cardiovascular disease. Aim of our study was to evaluate the prevalence of the metabolic syndrome and of its components in Type 2 diabetic patients.
**Materials and methods:** 1446 patients with Type 2 diabetes (age 62.1 ± 9.5 years; 59.8%M, 40.2%F; known duration of diabetes 7 years - range 1–44) have been examined during one day hospitalisation, in order to investigate the disorders featuring the metabolic syndrome. Clinical and laboratory data were collected by a computerized clinical data base „Eurotouch". The metabolic syndrome in a person with Type 2 diabetes is defined by the presence of at least two of the following disorders: waist circumference >102/88 cm (M/F); triglycerides ≥1.69 mmol/L; HDL-cholesterol ≤1.04/1.29 mmol/L (M/F); blood pressure ≥130/85 mmHg.
**Results:** Obesity (BMI> 30 kg/m$^2$) was present in a vast majority of patients (42%). Glycaemic control was stable ($HbA_1c$ 7.7 ± 1.3%). The proportion of patients with the metabolic syndrome was very high (n= 1370, 93.5%), with significant difference between males (91.9%) and females (95.8%) (chi-squared= 0.003). The average age was significantly higher in patients with the metabolic syndrome (62.3 ± 9.2 vs 59.1 ± 11.7 years, $p<0.05$). Most patients (87%) presented more than 2 additional abnormalities accompanying hyperglycaemia. Central obesity and low HDL-cholesterol were the most frequent association (39%) in patients with 2 components accompanying diabetes; among patients with 3 disorders besides hyperglycaemia, abdominal obesity, hypertriglyceridaemia and low HDL-cholesterol were the most frequent association (35%).

**Conclusion:** We confirm that the metabolic syndrome is prevalent in a significant proportion of type 2 diabetic patients. These data suggest that we need more intensive pharmacological treatment and lifestyle modifications to control this syndrome and its component conditions.

## 676

**Lifestyle modification for Type 2 diabetes**

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**Background and aims:** Exercise and improved diet are known to be beneficial in the management of type 2 diabetes mellitus. But in practice, it is difficult for us to predict the effect of education. We investigated differences between the lifestyle modification (LSM) responsive group and irresponsive group. And we determined the factor that influenced the responsiveness of LSM.
**Material and methods:** Sixty two Korean type 2 diabetic patient who were not treated previously participated in a lifestyle modification program. Patients received information and practical guidance regarding exercise and nutrition by nutritionist and exercise manager at each visit. Anthropometric measurements and blood sampling were checked at first and 3 months later. Diet and exercise habit and GXT were done at initial and 3 month later visit.
**Results:** Total patients were 62 (male:25, female:37), their mean age was 54 ± 9.1yr and mean BMI was 24.3 ± 3.1 kg/m$^2$. We divided the patients into 2 groups. Group 1 patients (n=42) were responsive to LSM and group 2 patients (n=20) were irresponsive, so they need oral hypoglycemic agents for glycemic control. In total patients, after LSM, weight (63.7 ± 10.3 vs. 62.7 ± 10.0 kg), BMI (24.3 ± 3.0 vs. 23.9 ± 2.9 kg/m$^2$), waist circumference (85.2 ± 8.3 vs. 82.5 ± 8.2 cm), waist-hip ratio (0.89 ± 0.05 vs. 0.86 ± 0.05) were significantly decreased, and fasting glucose (155.7 ± 41.3 vs. 121.6 ± 22.7 mg/dl), postprandial glucose (237.2 ± 81.1 vs. 154.0 ± 44.4 mg/dl), HbA1 c (8.1 ± 2.0 vs. 6.7 ± 1.7%) were also significantly improved. Patient's $VO_2$max (16.4 ± 3.3 vs. 18.2 ± 3.5) and heart rate reserve (0.25 ± 0.09 vs. 0.32 ± 0.08) were significantly increased. At initial examination, group 1 have higher waist circumference (65.5 ± 8.9 vs. 60.1 ± 12.2 cm), and waist-hip ratio (0.89 ± 0.04 vs. 0.86 ± 0.06) and lower fasting glucose (145.7 ± 29.4 vs. 176.7 ± 54.0 mg/dl) and HbA1 c (7.6 ± 1.7 vs. 8.7 ± 2.6%) than group 2. But there were no significant differences between 2 groups at 3 months later. Significant predictive factors for responsiveness to LSM were not found in logistic regression analysis. The frequency of group1 was significantly higher in patients who have good tolerance to education in Chi-Squrare analysis.
**Conclusion:** In Korean type 2 diabetic patients, LSM resulted in marked improvements in anthropometric measurements, glycemic control, and exercise capacity. The LSM responsive group had higher waist circumference, waist-hip ratio and lower fasting glucose and HbA1 c at initial state. The compliance to education influenced on the responsiveness of LSM. Therefore, we suggest that intensive lifestyle intervention can benefit the individual with type 2 diabetes.

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**Progressive resistance training (PRT) decreases abdominal fat and improves insulin sensitivity in Type 2 diabetic older men**

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Exercise training results in preferential loss of fat from the central regions. This loss of visceral adipose tissue is closely related to an improvement in insulin sensitivity. Aerobic endurance exercise has traditionally been advocated as the most suitable exercise mode in the treatment of patients with DM type 2. Recent studies have reported that PRT represents an adequate alternative to aerobic exercise; however, little is known about the optimal PRT regimen to improve insulin sensitivity.
The aim of our study was to evaluate the influence of two PRT sessions / week, without a concomitant diet, on abdominal fat and insulin sensitivity in type 2 diabetic older men.
**Methods :** Nine untrained, sedentary older men (mean age: 66,6 ± 3,1 yr) with DM type 2, without complications, participated in a 16 weeks PRT supervised program, two times per week. The subjects trained with loads of 50–80% of the individual 1 RM, 10–15/5–6 (higher loads) repetitions per set and 3–5 sets of each exercise. Baseline testing was completed twice during the previous four weeks of the study (weeks -4 and 0), at the eight week and at the end of the PRT program. Basal glycaemia was measured and insulin sensitivity was determined according to Bergman's minimal model procedure (FSIVGTT). Body mass and seven skin fold sites were measured. Abdominal fat was obtained by computer tomography. Energy intake was recorded for three days. Lower and upper body maximal strength was assessed using 1 RM actions in a half squat and in a bench press position respectively. A t-student test was performed between two series of values and significance was set at $p<0,05$.
**Results:** There were no changes in the parameters evaluated between the two baseline tests. After the 16 weeks training period, maximal leg (17,1%, $p<0,05$) and arm strength (18,2%, $p<0,001$) as well as caloric intake (15,5%, $p<0,05$) were increased. Significant decreases occurred in intraabdominal fat (–10,3%, $p<0,001$) and in the sum of skin fold thickness (–8,5%, $p<0,05$), whereas no changes were observed in body mass. Resistance training increased insulin sensitivity (+ 16,3%, $p<0,01$) and decreased fasting blood glucose (–7,1%, $p<0,05$). Glycosylated haemoglobin decreased at week eight (5,8 ± 1,2, $p=0,06$) but later increased again, so the final value (6,24 ± 0,9%) was similar to the initial value (6,23 ± 0,9%).
**Conclusion:** Twice weekly strength exercise, without a concomitant diet, improved insulin sensitivity and fasting plasmatic glucose, and decreased intraabdominal and subcutaneous fat in type 2 diabetic older men. These metabolic variations were presents in spite of the fact that body mass was unchanged and diary caloric intake was increased during the experimental period.

## 678

**The effect of long term exercise on intrahepatic lipid and the insulin sensitivity of glucose and fatty acid metabolism in sedentary male subjects**

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**Background and aims:** Adaptive changes in fatty acid metabolism, liver and muscle fat content and adipocyte-derived cytokines may potentially explain the beneficial effects of exercise on insulin action. We investigated this in sedentary men before and after 6 weeks of supervised exercise.
**Materials and methods:** Thirteen sedentary overweight male subjects (age 50 ± 3.4yr, BMI 28.2 ± 0.5) were recruited, seven were randomised to a 6 week exercise programme and six remained sedentary. After completion of the baseline (0 weeks) metabolic study and body composition measurements subjects who were allocated to the exercise group started the exercise programme. Subjects exercised at 60–85% of $VO_{2\,max}$ for a minimum of 20 minutes at least 3 times a week for 6 weeks. Insulin sensitivity of fatty acid (NEFA) production rate (Ra), glycerol Ra, glucose Ra and glucose disposal rate (Rd) were measured with stable isotopes of palmitic acid, glycerol and glucose at 0 and 6 weeks with a 2 step hyperinsulinaemic euglycaemic clamp (step 1, 0.3 (low dose); step 2, 1.5 (high dose) mU. kg$^{-1}$. min$^{-1}$). Intrahepatocellular lipid (IHCL) and intramyocellular lipid (IMCL) were measured by magnetic resonance spectroscopy and visceral fat by cross-sectional CT scanning.
**Results:** In the exercise group $VO_{2\,max}$ increased by 20 ± 5% after 6 weeks ($p<0.01$) but there was no significant change in the control group. There was no significant change in body weight or BMI in either group. After 6 weeks, the decrease in glucose Ra following low dose insulin was greater than at 0 week in the exercise group (3.8 ± 0.5 vs 2.8 ± 0.5 μmol.kg$^{-1}$.min$^{-1}$, 0 week vs 6 weeks, $p<0.05$) with no change in the control group (3.2 ± 0.7 vs 3.2 ± 0.4 μmol.kg$^{-1}$.min$^{-1}$). After 6 weeks the increase in the area under the curve for glucose Rd following high dose insulin was greater than at 0 week in the exercise group (5404 ± 735 μmol/kg vs 6508 ± 581 μmol/kg, $p<0.01$) with no change in the control group (4664 ± 538 μmol/kg vs 4628 ± 415 μmol/kg). In the exercise group there was a decrease in fasting NEFA concentration (0.81 ± 0.04 vs 0.62 ± 0.05 mmol/l, 0 week vs 6 weeks, respectively, $p<0.01$), fasting glycerol concentration (75 ± 8.3 vs 58 ± 6.6 μmol/l, $p<0.03$), fasting glycerol Ra (3.3 ± 0.8 vs 2.5 ± 0.6 μmol · kg$^{-1}$ · min$^{-1}$, $p<0.02$) and fasting NEFA Ra (4.5 ± 0.3 vs 3.7 ± 0.5 μmol · kg$^{-1}$ · min$^{-1}$, $p<0.04$), visceral fat content (186 ± 13 cm$^2$ vs 152 ± 10 cm$^2$, $p<0.04$) and fasting adiponectin (7.0 ± 0.9 μg/ml vs 5.8 ± 0.9 μg/ml, $p<0.05$) but no change in the control group. There was no change in IMCL in either group. The improved insulin sensitivity of glucose Ra in the exercise group correlated with a decrease in IHCL ($p<0.05$).
**Conclusion:** The improvement in the insulin sensitivity of lipolysis, results in decreased availability of circulating NEFA which in some subjects may lead to a reduction in IHCL content. This may contribute to the observed improvement in the insulin sensitivity of glucose Ra with exercise.
*This work was funded by the British Heart Foundation*

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# Exercise

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**Exercise training increases activity and mRNA expression of malonyl CoA decarboxylase in human muscle**

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**Background and aims:** An increase in the concentrations of malonyl CoA in muscle has been linked to insulin resistance in both rodents and humans. Presently we investigated, in healthy subjects, the effects of a long-term combined aerobic and dynamic strength training group program on malonyl CoA concentrations, AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) phosphorylation and protein content in skeletal muscle. In addition we determined malonyl CoA decarboxylase (MCD) activity and mRNA expression. Insulin sensitivity, substrate utilization and regional fat distribution were also measured.
**Materials and methods:** Eleven subjects with BMI between 22 and 32 kg/m$^2$, aged 51.0 ± 2.2 years participated in the study. Insulin sensitivity was measured by hyperinsulinemic euglycemic clamp, substrate utilization by indirect calorimetry, regional fat distribution by computerized tomography of abdomen and lean body mass (LBM) by x-ray bone densitometer (DXA). Basal and insulin-stimulated malonyl CoA concentrations, AMPK and ACC phosphorylation and protein content, as well as MCD activity and mRNA expression were determined in biopsies from the vastus lateralis muscle. All these investigations were performed before and after a 12-week combined aerobic and dynamic strength training group program.
**Results:** After exercise training, $VO_2$ max increased by 13% (45.7 ± 1.2 to 51.3 ± 0.8 ml/min/LBM, p=0.002) and intraabdominal fat area decreased with 15% (145 ± 15 to 123 ± 14, p=0.02). Training did not significantly influence glucose infusion rate (M-value), fasting levels of plasma free fatty acids (FFAs) or fasting substrate utilization rates. Insulin suppressed fat oxidation rates and plasma FFA levels both before and after training. After training, the concentration of malonyl CoA in muscle was decreased by 26% (0.19 ± 0.02 to 0.14 ± 0.01 nmol/g, p<0.05) and the MCD activity was increased by 88% (0.4 ± 0.02 to 0.75 ± 0.03 nmol/min/mg protein, p<0.001). Furthermore, mRNA expression of MCD in muscle was increased after training by 51% (0.55 ± 0.3 to 0.83 ± 0.1, p<0.05). Training did not influence protein content or phosphorylation of AMPK and ACC. During insulin infusion, malonyl CoA concentration was increased by 26% before and 29% after exercise training (p=0.03–0.01), and phosphorylation of ACC was decreased after training by 48% (p=0.03).
**Conclusion:** In conclusion, healthy middle-aged subjects participating in a 12-week exercise training program, without caloric restrictions, improved physical fitness and reduced intraabdominal fat mass, but did not increase insulin sensitivity. Notably, training decreased the concentration of malonyl CoA in skeletal muscle, and increased both activity and mRNA expression of MCD, while AMPK and ACC phosphorylation and protein content were unchanged.

## 680

**The effect of aerobic and anaerobic exercise according to Bruce protocol on apoptosis in Type 1 diabetes mellitus**

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**Background and aims:** Many studies demonstrated the effect of exercise on the immune system. The aim of this study was to investigate the effect of aerobic and anaerobic exercise on cellular immune system and apoptotic processes in type 1 diabetics.
**Materials and methods:** Patients with type 1 diabetes (n=19) and age-matched controls (n=20) were included in the study (Group 1, mean age 22.1 ± 2.8 and Group 2, mean age 22.3 ± 2.1). Group 1 had a disease duration of less than 5 years (3.37 ± 1.61) and $A_{1c}$ levels of ≤ 8%. In addition, diabetic patients were required to go through a normoglycemia period of at least 1 week prior to baseline. All patients exercised according to Bruce protocol. Glycemic changes, inspiration capacity, maximum ventilatory volume, max $Vo_2$, enduration time, resting heart rate, maximum heart rate, heart rate after 3 minutes of rest were determined both before and after the exercises, lymphocyte surface molecules CD3, CD4, CD8; apoptotic activity (CD95 Fas/APO-1) were investigated by Flow Cytometry.
**Results:** Glucose levels before and after exercise were 151.9 ± 31.7 and 133.1 ± 36.0 mg/dl in Group 1 (p=0.012) and 91.7 ± 9.9 and 98.4 ± 17.5 mg/dl in Group 2; the difference among groups was insignificant. However, the difference between both groups with respect to test results before and after exercise was significant (p<0.001 and p=0.001, respectively). Inspiration capacity was 3.2 ± 0.6 L in Group 1 and 3.6 ± 0.6 in Group 2 (p=0.040). Maximum ventilatory volume (L/min) was 152.7±29.1 L/min in Group 1 and 177.9±31.4 L/min in controls (p=0.014). Max $VO_2$ (%) was 46.2±8.2 Group 1 and 59.3 ± 8.1 in Group 2 (p<0.001). During the exercise tests, the enduration time (exercise termination time; min) was 10.0 ± 0.9 in Group 1 and 11.9 ± 1.6 in Group 2 (p<0.001).
CD3 before and after the exercise were 66.3 ± 7.2 and 59.3 ± 10.0 in Group 1; and 67.4 ± 10.0 and 62.7 ± 9.0 in controls (p=0.011). T helper cells, CD4, before and after the exercise were 35.5 ± 6.9 and 27.6 ± 8.1 (p<0.001) in Group 1 and 36.5 ± 9.5 and 27.2 ± 6.2 in Group 2 (p<0.001). Cytotoxic T lymphocytes, CD8 before and after the exercise were 31.4 ± 7.6 and 32.7 ± 9.4 in Group 1 (p=0.390) and 32.2 ± 8.3 and 36.2 ± 5.3 in Group 2 (p=0.021). CD95 before and after the exercise were 38.0 ± 15.2 and 49.4 ± 15.3 in Group 1 (p<0.001) and 29.9 ± 14.1 and 35.7 ± 16.5 in Group 2 (p=0.012).
**Conclusion:** Our study suggests that the magnitude of suppression on cellular immunity by exercise may linearly correlate with the intensity of exercise. These findings demonstrate an increase in apoptotic activity after the exercise. Therefore exercise of moderate intensity can be recommended for type 1 diabetic patients to maintain immunological functions at an optimum level and to prevent suppression of immunity.

## 681

**The impact of walking to school on overall physical activity and metabolic health**

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**Background and aims:** The last decade has seen a parallel rise in the prevalence of childhood obesity and diabetes. Physical inactivity is assumed to be a key contributory factor and concern has been expressed about the increasing use of motorised transport to and from school. Indeed, government-backed schemes, such as 'walking buses', have been widely implemented in order to encourage more young children to walk to school in safety. We have analysed the impact of walking to school on overall physical activity, body mass and metabolic health in young children from the Early-Bird study.
**Materials and methods:** We used MTI (formerly CSA) accelerometers to monitor the physical activity of 275 school children (121 girls, 154 boys, mean age 4.9y), attending 50 different city primary schools, for 7 consecutive days (5 school days and a weekend). Questionnaires were used to determine whether the child walked to school, and the time taken. Physical activity, body fat (BMI and sum of five skin-fold thicknesses) and metabolic status (insulin resistance by HOMA and fasting triglycerides) were compared between children who walked to school (W) and those who were driven by car (C). Numbers were sufficient to demonstrate, with 80% power, a significant difference in school journey activity between W and C of 12%.
**Results:** Twice as many children walked to school as were driven. The median distance to school was 0.7 km (IQR 0.4–1.2 km), and the median walking time 6 minutes (5–10 min). In 82% of cases the walk to school took less than 15 minutes, identical to the national UK figure. Physical activity recorded during the 10 journey periods to and from school was 18% higher among those who walked (W 4.90, C 4.15 units, p<0.001). However, this difference represented only 2% of the mean total weekly activity, and made no difference to it (W 37.56, C 37.60 units, p=0.97). There were no differences in BMI (W 16.1, C 16.2 kg/m$^2$, p=0.88), skin-folds (W 4.10, C 3.93 cm, p=0.28), insulin resistance (W 0.76, C 0.71 units, p=0.41) or triglyceride levels (W 0.57, C 0.60 mmol/l, p=0.27) between W and C. The patterns were the same whether gender, social class or school term were analysed separately or together.

**Conclusion:** This study challenges a widely held perception that walking to school will help reverse or even stem the rise in childhood obesity. In reality, in five-year-old children, 1) walking to the neighbourhood primary school makes no significant contribution to their overall physical activity. These data do not support the adverse publicity given to motorised transport to school, nor the public's perception of its impact. 2) going to school by car is unlikely, at this age, to be detrimental to children's health. There may be other benefits from walking to school (a reduction in traffic and noise pollution), but metabolic health does not appear to be one of them.
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**Effect of exercise on immun parameters in Type 1 diabetes**
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**Background and aims:** The aim of the study was to compare respiratory functions and metabolic parameters and to evaluate the changes in the percentages of T and B lymphocytes, and NK cells; and also to demonstrate changes in activation markers of immune system caused by a standart exercise protocol (Bruce) in type 1 diabetes patients and control subjects.
**Materials and methods:** Diabetic Group consisted of 19 Type 1 diabetic patients with 16–25 years of age and disease duration less than 5 years and also having $HbA_{1c}$ values < 8%. Age-matched male healthy subjects (n=20) was included as Control Group in to study. None of the subjects had any infections detected in previous 2 weeks.
**Results:** Comparing respiratory functions and metabolic parameters of both groups, no statistical difference was found in heart rates, at the beginning, maximal status and resting at the 3rd minute after the exercise. Inspiration capacity, max. respiratory volume, max. $VO_2$ and exercise time were found to be statistically lower in type 1 diabetics (p=0.040, p=0.014, p<0.001 and p<0.001, respectively). Mean blood sugar before exercise was calculated 152 ± 31 mg/dl in type 1 diabetics and was decreased to 133 ± 36 mg/dl after the exercise (p=0.012).
At the beginning of the exercise CD3, CD4, CD8 and NK percentages and CD4/CD8 ratio were similar in both groups, but CD19 and CD23 were higher in diabetic group before and post exercise levels. After the exercise; CD3, CD4 levels and CD4/CD8 ratios were found to be lower in contrast to be higher levels of NK cells in both groups (p<0.001). In addition, CD25 levels and CD25/CD3 ratios were higher in diabetics, but CD23/CD19 ratios were observed to be higher in control subjects.
**Conclusion:** Submaximal aerobic exercise because of its no negative effects on the immune system may be recommended for type 1 diabetics without any metabolic and respiratory complications.

## 683

**Effect of leisure-time physical activity on glycaemic control, insulin dose and insulin sensitivity in Type 1 diabetes mellitus**
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**Background and aims:** In type 1 diabetes, studies have failed to show an effect of physical activity on glycaemic control. These studies are, however, limited by small numbers of patients or semi-quantitative assessment of physical activity. In type 2 diabetes, on the other hand, glycaemic control has been convincingly shown to improve by physical activity. Therefore, or aim was to investigate the effect of leisure-time physical activity (LTPA) on glycaemic control, insulin dose and insulin sensitivity in a large cohort of type 1 diabetic patients.
**Material and methods:** This is a cross-sectional study of 624 type 1 diabetes patients (M/F: 276/348) participating in the ongoing FinnDiane-study with an age of 37 ± 12 yrs (mean±SD) and duration of diabetes 20 ± 12 yrs. The patients had normal albumin excretion rate (<30 mg/24 h) and were free of coronary heart disease, peripheral vascular disease and stroke. LTPA was assessed by a validated and quantitative questionnaire and energy expenditure was expressed as METh/week. Patients were classified as sedentary (<10 METh/week), moderately active (10–40 METh/week) and active (>40 METh/week). Two hours of walking per week corresponds to 10 METh/week. As a measure of insulin sensitivity we used estimated glucose disposal rate (eGDR), an estimate well correlated with values measured with a euglycaemic clamp. ANOVA or Kruskal-Wallis tests were used in analyses and P-values were corrected for age and BMI.
**Results:** The $HbA_{1c}$ of sedentary, moderately active and active females was 8.7 ± 1.4, 8.2 ± 1.3 and 8.2 ± 1.3% (P=0.042), respectively. In males, the corresponding values were 8.3 ± 1.3, 8.1 ± 1.4 and 8.1 ± 1.2% (P=NS). logLTPA correlated with $HbA_{1c}$ in females (r =–0.14, P=0.008) but not in males (r =–0.04, P=NS). In the lower 50$^{th}$ percentile of LTPA, the correlation was –0.18 (P=0.016) in females, while no correlation was found in the higher 50$^{th}$ percentile. Insulin doses were 0.76 ± 0.21, 0.74 ± 0.23 and 0.69 ± 0.23 IU/kg/24 h (P=0.013) in males, while females had 0.72 ± 0.20, 0.72 ± 0.24 and 0.67 ± 0.20 IU/kg/24 h (P=NS), respectively. eGDR was (median [IQR]) 7.1 [4.6–8.5], 7.8 [5.6–9.0] and 7.9 [5.9–9.2] (P=0.006), respectively, with no major gender difference.
**Conclusions:** Higher levels of LTPA were associated with better glycaemic control in female and lower insulin dose in male type 1 diabetic patients. More physically active patients had a better insulin sensitivity than less active patients. The positive effect on $HbA_{1c}$ was most apparent in the lower range of LTPA while high levels of LTPA had no additional effect. However, the causal relationships and clinical significance of the findings should be investigated in future longitudinal studies.
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## 684

**Lycopene protective effect on oxidative stress generation during physical exercise in patients with Type 1 diabetes**
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ADA has recently considered the role of physical exercise also in type 1 diabetic patients, for cardio-vascular complications prevention. However physical activity can generates free radicals and depletion of antioxidant defences. So in sedentary diabetic patients, already exposed to oxidative stress for high glycemic values, it is important to increase antioxidant defences.
**Aim of the study:** We evaluated if supplementation with lycopene, a powerful natural antioxidant contained in tomato juice, is protective for antioxidant defences in type 1, sedentary, young diabetic patients submitted to moderate physical exercise-derived oxidative stress.
**Materials and methods:** 6 sedentary diabetic patients (4 males and 2 females), age included between the 19 and 31 years, performed two identical physical exercises (15 minutes of race with moderate pace of about 9 km/hour) at distance of at least 2 days between the two race. We evaluated glycaemia and TRAP (total radical antioxidant parameter) before and after the race. Three hours before second race the subjects took 250 ml of tomato juice. Preliminary in healthy subjects we verified that the lycopene increases antioxidant defences gradually in 3 hours after assumption.
**Results:** Glycemic values pre and post race were similar (first race: mean glycemia pre-race 150 ± 40,85 mg/dl, (Mean ± DS); post-race 145,33 ± 39,54 mg/dl. 2nd race: pre-race 166,67 ± 58,37 mg/dl; post-race 166,83 ± 77,04 mg/dl). Basal TRAP values before physical exercise were greater after tomato juice assumption (343,5, ± 68,63 mmol HClO/ml vs. 473,17 ± 53,21 mmol HClO/ml; p<0.005). Physical exercise reduced in both the cases the levels of TRAP (without lycopene: 343,50 ± 68,63 mmol HClO/ml vs. 247,17 ± 46,38 mmol HClO/ml; p<0.05. After lycopene: 473,17 ± 53,21 mmol HClO/ml vs. 347,67 ± 64,88 mmol HClO/ml; p<0.05). TRAP values after physical exercise maintained higher after tomato juice assumption (247,17 ± 46,38 mmol HClO/ml vs. 374,67 ± 64,88 mmol HClO/ml; p<0.005). The loss of TRAP in two exercises was similar (D of variation: –98,50 ± 26,83 vs. –96,33 ± 52,90; p n.s.)
**Conclusions:** Lycopene assumption seems to be protective during moderate physical exercise-derived oxidative stress in type 1 sedentary diabetic patients.

## 685

**Identifying improvements in exercise-induced glucose tolerance in individuals with Type 2 diabetes by continuous glucose monitoring**

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**Background and aims:** Exercise has become a cornerstone treatment in the management of Type 2 diabetes, principally because of its positive effect on glycaemic control. We have previously demonstrated that moderate exercise increases glucose removal in individuals with Type 2 diabetes. Our aim was to study the effect of moderate intensity exercise on short (< 2 hours) and long term (~72 hours) glycaemic control using a Continuous Glucose Monitoring System (CGMS, MiniMed Medtronic).
**Materials and methods:** Six sedentary, diet-treated patients (age 61.2 ± 8.1yr; body mass 101.1 ± 19.0 kg) with Type 2 diabetes were monitored using the CGMS for ~36 hours prior to and ~36 hours post exercise. Subjects we studied on two consecutive days at ~08.30 having fasted for 12 hours. CGMS values and forearm venous blood glucose responses to an Oral Glucose Tolerance Test (OGTT) at rest and immediately following one hour of moderate intensity (90% of a predetermined lactate threshold) exercise were recorded. Venous blood was sampled every 10 minutes and immediately analysed for glucose and lactate concentrations.
**Results:** All individuals had been previously diagnosed with Type 2 diabetes by a General Practitioner and demonstrated fasting venous glucose concentration >7 mmol/l and/or venous glucose >11.1 mmol/l 2 hours after an intake of 75g of glucose. Exercise was moderate in intensity, as lactate concentrations at the end of exercise did not exceed pre-determined lactate threshold values (2.66 ± 0.69 vs. 3.4 ± 1.1 mmol/l). Whole day average glucose concentrations were significantly lower on the day following exercise, when compared with the day prior to exercise (9.7 ± 3.1 vs.11.8 ± 3.5 mmol/l $p<0.05$). Chronic effects on glycaemic control were shown by a increase in the time period through a day when glucose concentration remained below 13 mmol/l. The total time that glucose concentrations spent below 13 mmol/l increased by 18% on the day following the exercise ($p<0.05$). The venous blood glucose ($k = -2.4 \pm 1.2$ vs. $-0.74 \pm 0.67 \times 10^{-3}$ $min^{-1}$) and CGMS ($k = -2.9 \pm 0.5$ vs. $-0.9 \pm 0.3 \times 10^{-3}$ $min^{-1}$) calculated rate constant for glucose decay from peak glucose concentration during the OGTT was greater immediately following than the day before the exercise ($p<0.05$).
**Conclusion:** Moderate exercise increased the rate of glucose removal and improved glycaemic control in individuals with Type 2 diabetes. The Continuous Glucose Monitoring System is an accurate and simple method of measuring acute and chronic exercise-induced changes in glycaemic control in individuals with Type 2 diabetes.
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## 686

**Impact of aerobic exercise training on insulin sensitivity, aerobic and muscle oxidative capacity in offspring of Type 2 diabetic patients**

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**Background and aims:** As first degree relatives (offspring) of patients with type 2 diabetes have been shown often to be insulin resistant and to have decreased $VO_2$max the metabolic and cardiovascular response to exercise training could be different from what is expected in subjects without diabetic predisposition.
**Materials and methods:** Twenty-nine healthy and glucose tolerant offspring (age 33 ± 5 y; BMI 26.3 ± 1.6 kg/m²) and 19 matched control subjects (age 31 ± 5 y; BMI 25.8 ± 3.0 kg/m²) completed a bicycle ergometer exercise program for 10 weeks. Before and after, study subjects were assessed by insulin sensitivity, $VO_2$max, body composition and skeletal muscle citrate synthase (CS) and cytochrome c oxidase (COX) enzyme activities.
**Results:** Both groups showed a substantial improvement of comparable magnitude in aerobic capacity (offspring: 51.5 ± 7.9 vs. 59.3 ± 9.9 ml/kg FFM/min (14.1 ± 11.3%); $P < 0.001$; and controls: 52.5 ± 7.8 vs. 60.7 ± 10.3 ml/kg FFM/min (16.1 ± 14.2%); $P < 0.01$. Insulin sensitivity was also improved in equally in offspring (7.4 ± 2.6 vs. 8.2 ± 2.9 mg/kg FFM/min; $P = 0.06$) and in control subjects (9.1 ± 2.9 vs. 10.2 ± 3.6 mg/kg FFM/min; $P = 0.07$). At baseline, activities of the oxidative muscular enzymes COX and CS were reduced in offspring (n = 22) compared to controls (n = 16); COX: 43.4 ± 11.6 vs. 49.4 ± 11.6 units/min; $P = 0.12$ and CS: 28.0 ± 6.0 vs. 31.7 ± 6.0 units/min; $P = 0.07$. In response to the exercise program both groups demonstrated a significant increase of comparable magnitude in both COX and CS enzyme activities; offspring: $P < 0.001$ (both enzymes); controls: $P < 0.01$ (both enzymes).
**Conclusion:** The cardiovascular and metabolic response to exercise are preserved in prediabetic subjects and comparable to the magnitude of change in subjects without predisposition to type 2 diabetes. Baseline activities of the oxidative enzymes CS and COX are decreased but the ability to enhance the activities of these enzymes by exercise is not impaired.

## 687

**Effects of the passive physical exercise on the metabolic control and on the arterial pressure in Type 2 diabetic patients**

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**Background and aims:** The physical exercise represents a primary intervention for a correct approach to the diabetic patient. This study evaluates the effects of a passive physical exercise on the glycaemia and on the arterial pressure in type 2 diabetic patients.
**Materials and methods:** The study has been conducted on 300 type 2 diabetic patients of middle age 61.1 ± 10.2 years, with duration of the illness of 11.4 ± 10.5 years, without chronic complications. All the patients, in poor metabolic control ($HbA_{1c}$ 7.8 ± 0.2%) and in treatment with oral hypoglycaemic agents have been divided in two groups, the first one with BMI < 30 Kg / m² (27.6 ± 1.4 Kg / m²) and the second one with BMI > 30 Kg/m² (34.3 ± 4.7 Kg/m²s). Both groups effected, in different days, three tests using the NEMES platform (Neuromuscolar Mechanical Stimulator by Bosco). The NEMES consists in a platform that determine a vertical vibration at predetermined frequencies (30 Hz) with a periodic duration of the vibratory stimulus. Each test consists of a series of five repetitions each one of one minute. Between the first and the second test there was an interval of 5 minutes and between the second and the third one of 30 minutes. The first day the platform was switched off, as a placebo effect while the second day it was switched on.
**Results:** Before the study and at the end of the three tests the glycaemia and the arterial pressure has been evaluated. In the first group (BMI < 30 Kg/m²), with the switched off platform, the glycaemia was not modified. Instead, with the switched on platform a glycaemic reduction was statically significant both after 5 min from the beginning of the test ($p < 0.005$) and after 30 min ($p < 0.002$). In the other group (BMI > 30 Kg/m²), with the switched off platform, the glycaemia was not changed while it was observed a marked reduction of it both after 5 min from the beginning of the test ($p < 0.0004$) and after 30 min ($p < 0.0001$). Also it was observed a significant reduction of the arterial pressure in both groups after 30 min of the beginning of the test ($p < 0.05$).
**Conclusion:** The results of this study have shown, through a vibration of the adapted wavelength of short time but of elevated intensity, a reduction of the glycaemia and the arterial pressure mostly in obese subjects (probably due of an improvement of the insulin resistance and/or better ability of adaptation to the vibratory stimulus of the pressoreceptors). Such passive physical exercise could represent, in the short term, the first step to a correct and sure practice of the physical activity and an effective motivation for type 2 diabetic patients with the aim to define therapeutic goals that guarantee an improvement of the quality of life through a reduction of the diabetic complications.

## 688

**Energy balance in Bangladeshi Type 2 diabetic patients with hypercholesterolemia**

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**Background and aims:** Determining energy balance is an important step towards providing customized dietary advice to newly diagnosed diabetic patients. There is no published data on energy balance in Bangladeshi population. In the above context, this study was undertaken to determine the energy intake and expenditure in newly diagnosed diabetic subjects with hypercholesterolemia and to observe their relation with anthropometric

parameters and some biochemical variables (plasma glucose and serum total cholesterol).
**Materials and methods:** Ninety seven newly diagnosed type 2 diabetic subjects (male:female 61:36, age 46 ± 9 years, BMI 24 ± 5, mean±SD) with hypercholesterolemia (fasting plasma total cholesterol >200 mg/dl) were selected from the Out-Patient Department of BIRDEM (the central institute of the Diabetic Association of Bangladesh with around 2500 patient attendance per day) by random sampling. The daily total energy intake was calculated from dietary history using 24 hr recall method. The total daily energy expenditure of the subjects was calculated by a factorial method using Physical Activity Level (PAL). Energy balance was calculated by subtracting the total daily energy expenditure from the total daily energy intake.
**Results:** In 87 (89%) subjects the waist-to-hip ratio was high (i.e male ≥ 0.90 and female ≥1.0). On the basis of BMI, 50 (52%) subjects were normal, 6 (6%) were Chronic Energy Deficient (CED), 34 (35%) were overweight, and 4 (4%) were obese. The median (range) energy intake of the subjects was 1691 (1018–3076) kcal/day. In 16 (17%) subjects this intake was 300 kcal more than the recommended amount. All the subjects were of light PAL. The median (range) duration of exercise of the subjects was 45 (0–120) min/day. The physical exercise was adequate in duration only in 21 (22%) subjects. The median (range) energy expenditure of the males and females were 2495 (1293–2908) and 2087 (1836–3308) kcal/day respectively. Total energy expenditure significantly correlated with fasting plasma glucose (r= –0.27, P=0.008) and with 2 h post glucose load plasma glucose (r= –0.33, P= 0.008). There was no correlation between total daily energy expenditure and serum total cholesterol. Overall positive energy balance was found in 6 (6%) subjects all of whom were of normal BMI; negative energy balance was found in 88 (94%) subjects. In normal BMI 6 (6%) subjects was found positive energy balance and 44 (46%) was found negative energy balance. In CED, over weight, and obese all the subjects were in negative energy balance.Energy balance was acceptable in 44 (46%). (6 (13%) normal BMI, 4 (9%) over weight, 34 (77%) obese). The daily energy balance significantly correlated with fasting plasma glucose (r=0.36, P<0.0001) and 2 h post glucose load plasma glucose (r=0.32, P=0. 011). No correlation was found between energy balance and serum total cholesterol.
**Conclusion:** Newly diagnosed type 2 diabetic subjects in Bangladesh with hypercholesterolemia perform light physical activity. Higher daily energy expenditure is associated with better glycemic status, but not with serum total cholesterol. Around 1/10 th of diabetic subjects with hypercholesterolemia on initial diagnosis have positive energy balance which is correlated with poor glycemic status.

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# Anti-obesity drugs

## 689

**The effect of sibutramine on insulin secretion in obese Type 2 diabetic patients and non-diabetic subjects**
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**Background and aims:** The aim of the present controlled, randomized, open-label study was to evaluate the effect of sibutramine combined with hypocaloric diet on body weight, body fat mass and insulin secretion in obese type 2 diabetic patients and nondiabetic subjects.
**Materials and methods:** 53 diabetic patients, of mean age 46.2 ± 7.4 years, mean BMI 33.9 ± 2.5 kg/m$^2$ and 59 nondiabetic subjects of mean age 43.2 ± 5.7 years and mean BMI 34.7 ± 2.9 kg/m$^2$ were treated with sibutramine at a mean dose of 12.8 ± 2.9 mg for three months. 42 age- and BMI-matched type 2 diabetic patients and 50 nondiabetic subjects only on hypocaloric diet were also followed-up and served as control groups. All the patients maintained their initial antidiabetic therapy (drug and dose) till the end of the study. The percentage of body fat mass was measured by means of an impedance technique (Omron, USA). Phases of insulin secretion (first – FPIS and second – SPIS) were studied during IVGTT. The area under the curve (AUC) was calculated for the total insulin secretion during IVGTT.
**Results:** We have found a significant reduction in body weight in both sibutramine-treated groups - type 2 diabetic patients - 6.8% vs 2.9% in the control group ($p<0.001$) and nondiabetic subjects - 8.7% vs 2.9% in the control group ($p<0.0001$). This was accompanied by a significant reduction in body fat mass in both treated groups ($p<0.0001$). We have established a significant decrease in waist circumference in both treated groups - type 2 diabetic patients ($p<0.01$) and nondiabetic subjects ($p<0.0001$) as compared to the corresponding control groups. Weight loss was accompanied by an increase of 43.8% in FPIS in the sibutramine-treated diabetic group ($p<0.0001$ as compared to the control group). There were no significant changes in the SPIS and AUC for total insulin secretion during IVGTT following sibutramine therapy in the diabetic subjects. In contrast to that, in the sibutramine-treated nondiabetic subjects there was a decrease in FPIS ($p<0.0001$), SPIS ($p<0.001$) and AUC for total insulin secretion ($p<0.0001$) at the third month.
**Conclusion:** The results of this controlled, randomized, open-label study demonstrate that sibutramine leads to a significant reduction in body weight, body fat mass and waist circumference in obese subjects. Sibutramine increases FPIS in type 2 diabetic patients and decreases overall insulin secretion in obese nondiabetic subjects. Thus sibutramine appears to be an effective drug in the treatment of obesity in both diabetic and nondiabetic subjects.

## 690

**Efficacy of orlistat plus lifestyle changes in risk reduction of Type 2 diabetes in obese patients with metabolic syndrome – a comparative analysis using national cholesterol education program Adult Treatment Panel III *vs* European group for the study of insulin resistance criteria**
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**Background and aims:** Patients (pts) with metabolic syndrome (MS) are at increased risk of developing type 2 diabetes (T2D). We assessed the effects of orlistat (ORL) plus lifestyle changes on the progression to T2D in obese patients with MS (as defined by the ATP III and the European Group for the Study of Insulin Resistance [EGIR]) in the XENical in the prevention of Diabetes in Obese Subjects (XENDOS) study.
**Materials and methods:** This was a retrospective analysis of the subset of pts with MS in a large, randomised, double-blind, placebo (PLA)-controlled trial of the effects of ORL plus lifestyle changes on the progression to development of T2D in obese pts (BMI ≥30 kg/m$^2$). Study participants had NGT (2-hour whole blood glucose <10 mmol/L and fasting whole blood glucose <6.7 mmol/L) or IGT (2-hour whole blood glucose 6.7–10 mmol/L and fasting whole blood glucose <6.7 mmol/L) at baseline. Pts received ORL 120 mg (n=1650) or PLA (n=1655), tid for 4 years together with a reduced-calorie diet (~800 kcal/day deficit) and encouragement to walk at least 1 extra kilometre a day. ATP III criteria for MS were ≥3 of: waist circumference >102 cm (men) or 88 cm (women); triglycerides ≥1.7 mmol/L; HDL-choles-

terol <1 mmol/L (men) or <1.3 mmol/L (women); BP ≥130/85 mmHg; fasting plasma glucose ≥6.1 mmol/L. An additional and comparative analysis using the EGIR criteria for MS was performed: hyperinsulinaemia (single 2-hour whole blood glucose >65 pmol/L) plus 2 of the following: elevated BP (systolic BP ≥140 mmHg and/or diastolic BP ≥90 mmHg), waist circumference (≥94 cm for men, ≥80 cm for women), fasting plasma glucose (≥6.1 mmol/L) or dyslipidaemia (plasma triglycerides >2.0 mmol/L and/or HDL-cholesterol <1.0 mmol/L).
**Results:** ATP III-defined MS was present in 1320/3277 (40%) of the intent-to-treat population (ORL n=648, PLA n=672). ORL plus lifestyle changes achieved significantly greater mean weight loss than PLA plus lifestyle changes over 4 years of treatment (−6.33 *vs* −2.96 kg; p<0.001). After 4 years, the cumulative incidence of T2D in the MS subgroup was significantly lower in the ORL group than the PLA group (9.8% *vs* 13.7%; p=0.03). ORL was associated with a 35.8% reduction in risk of progression to T2D compared with PLA. ORL treatment was also associated with significant differences in the change from baseline at year 4 for fasting whole blood glucose (0.08 *vs* 0.023 mmol/L, p<0.01), systolic BP (−5.48 *vs* −3.36 mmHg, p<0.05), diastolic BP (−3.31 *vs* −1.80 mmHg, p<0.05), LDL-cholesterol (−0.43 *vs* −0.15 mmol/L, p<0.001) and LDL:HDL ratio (−0.62 *vs* −0.40, p<0.001). EGIR-defined MS was present in 1394/3277 (42.5%) of intent-to-treat pts (ORL n=694, PLA n=700). Analysis of these pts showed the cumulative incidence of T2D after 4 years was 10.2% in the ORL group and 15.2% in the PLA group (p=0.0091); ORL was associated with a 40.0% risk reduction in pts with EGIR-defined MS.
**Conclusion:** Long-term treatment with ORL plus lifestyle changes reduces the risk of developing T2D by 36–40% in obese pts with MS defined by either ATP III or EGIR criteria, respectively. This retrospective analysis is the first to demonstrate that the progression to T2D can be prevented with ORL in pts with MS, independent of how MS is defined.
*Supported by: F. Hoffmann-La Roche*

## 691

**Orlistat improves glycaemic control and weight loss in overweight and obese Chinese patients with newly diagnosed and previously untreated Type 2 diabetes**
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**Background and aims:** The clinically beneficial effects of orlistat (ORL) in patients with type 2 diabetes (T2D), treated concomitantly with oral antidiabetic medications, have been demonstrated in previous studies. This is the first study to investigate the efficacy of ORL, in combination with a mildly reduced-calorie diet, in enhancing glycaemic control and promoting weight loss in overweight and obese patients with newly diagnosed and previously untreated T2D.
**Materials and methods:** Overweight and obese Chinese adults (BMI 25-<40 kg/m$^2$; aged 18–65 years) with a diagnosis of T2D in the previous 6 months were recruited at 12 centres in China and randomised to 6 months' double-blind treatment with ORL 120 mg (n=125) or placebo (PLA) tid (n=124), in combination with a reduced-calorie diet (−400 kcal/day). Diagnosis of T2D was made on the basis of fasting plasma glucose (FPG) ≥7.0 mmol/L or 2-h post-glucose OGTT ≥11.1 mmol/L, plus $HbA_{1c}$ 6.5–8.5%.
**Results:** After 6 months, the reductions in $HbA_{1c}$ were significantly greater in the ORL treatment group compared to the PLA treatment group (−0.96 ± 1.03% *vs* −0.55 ± 0.94%; p<0.001). More ORL- than PLA- treated patients had a decrease in $HbA_{1c}$ of ≥0.5% (65.3% *vs* 54.5%; p=NS) and ≥1.0% (46.8% *vs* 31.7%; p<0.05). Mean (± SD) reductions in both FPG and 2-h post-glucose OGTT were also significantly greater with ORL-than with PLA-treated patients (−1.15 ± 1.72 mmol/L *vs* −0.37 ± 1.73 mmol/L [p<0.001] and −4.05 ± 4.09 mmol/L *vs* −1.37 ± 4.40 mmol/L [p<0.0001], respectively). In addition, a greater proportion of ORL-treated patients (44%) had improved from T2D to normal glycaemic control or IGT compared with PLA-treated patients (33%) after 6 months. These improvements in glycaemic control were accompanied by improvements in blood pressure and lipid profile. In addition, after 6 months, the mean (± SD) reductions in body weight were significantly greater in the ORL treatment group compared with the PLA treatment group (−5.16 ± 3.37 kg *vs* −2.13 ± 3.15 kg; p<0.0001). Furthermore, more than twice as many ORL-than PLA-treated patients lost ≥5% of their initial body weight (60.5% *vs* 26.8%; p<0.0001), and approximately four times as many ORL- than PLA-treated patients lost ≥10% of their initial body weight (20.2% *vs* 4.9%; p<0.001). ORL was well tolerated and had a similar tolerability profile to placebo, with the exception of gastrointestinal adverse events, as seen previously.
**Conclusion:** Treatment with ORL, in combination with a mildly reduced-calorie diet, significantly improved and normalised glycaemic control, and improved weight loss in overweight and obese Chinese patients with newly diagnosed, previously untreated T2D. This study is the first to demonstrate these improvements within this patient population.
*Supported by: F. Hoffmann-La Roche*

## 692

**Orlistat enhances weight loss and improves cardiovascular risk factors in overweight and obese patients with Type 2 diabetes**
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**Background and aims:** Previous studies have demonstrated that even modest weight loss can result in clinically meaningful reductions in obesity-related risk factors and improvements in the control of associated diseases, particularly type 2 diabetes (T2D). The aim was to assess the clinical benefits of orlistat (ORL) treatment in overweight and obese patients with T2D on glycaemic control, weight loss, and other obesity-related risk factors.
**Materials and methods:** Pooled data from 5 randomised, multi-centre, double-blind, placebo (PLA)-controlled studies were retrospectively analysed. Patients (BMI 28–50 kg/m$^2$) were randomised to either ORL 120 mg (n=1418) or PLA (n=1274), both tid, in conjunction with a mildly calorie-reduced diet in studies of either 24 or 52 weeks. Of this total patient population, 366 of patients randomised to ORL and 336 patients randomised to PLA had T2D. Patients with T2D were allowed to receive oral hypoglycaemic medications either prior to and/or after randomisation. All data are least squares mean changes from baseline to study endpoint (last observation carried forward; intent-to-treat population consists of: n=362 for ORL group, n=332 for PLA group).
**Results:** ORL-treated T2D patients had significantly greater reductions in weight, and significantly greater improvements in glycaemic control and cardiovascular risk factors, as shown in the table below[a]. As well as significantly greater reductions in $HbA_{1c}$, 50% more ORL-treated patients than PLA recipients achieved $HbA_{1c}$ reductions of ≥0.5% (37.6% *vs* 23.5%; p<0.0001) and twice as many ORL-treated patients achieved reductions of ≥1.0% compared with PLA recipients (22.1% *vs* 10.5%; p<0.0001). At endpoint, 24.2% of ORL-treated patients achieved normal glucose tolerance compared with 14.2% of placebo recipients (p=0.0003).
**Conclusion:** In overweight and obese patients with T2D, treatment with ORL, in conjunction with a mildly calorie-reduced diet, resulted in significant improvements in glycaemic control, clinically meaningful weight loss, and improved cardiovascular risk factors.

(aEndpoint data from pooled 24 and 52 week studies)

| Variable | ORL | PLA | p value |
|---|---|---|---|
| Fasting plasma glucose (mmol/L) | −1.322 | −0.418 | <0.001 |
| $HbA_{1c}$ (%) | −0.433 | −0.030 | <0.001 |
| Weight (kg) | −4.767 | −1.690 | <0.001 |
| Systolic blood pressure (mmHg) | −6.597 | −4.462 | 0.070 |
| Diastolic blood pressure (mmHg) | −5.013 | −3.372 | 0.024 |
| Change in total cholesterol (%) | −4.063 | −1.111 | 0.009 |
| Change in LDL-cholesterol (%) | −5.334 | +0.016 | 0.003 |
| Change in LDL:HDL ratio (%) | −2.626 | 1.237 | 0.112 |

*Supported by: F. Hoffmann-La Roche Ltd*

## 693

**Protective effects of beta3-adrenoceptor agonist on insulin secretion in an animal model of Type 2 diabetes**
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**Background and aims:** There are many reports that beta3-adrenoceptor (beta3-AR) agonist reduced plasma glucose in type 2 diabetic rodent models. Beta3-AR agonists convert large adipocytes into small adipocytes, and it is associated with the amelioration of insulin resistance. On the other hand, there are reports that beta3-AR agonists increase insulin secretion. In the present study, we examined the effects of selective beta3-AR agonist

(KTO-7924: KTO) on insulin secretion in a model of type 2 diabetes that have been decreased insulin sensitivity and declined insulin secretion.
**Materials and methods:** Male C57BL/KsJ db/db mice and db/+m mice were used. KTO (10 mg/kg, p.o., twice a day) and vehicle were administered for 28 days from 6-weeks of age. On day 7, 14, 21, 28, blood samples were obtained from the retro-orbital sinus of mice under feeding condition and plasma insulin, HbA1 c and plasma glucose were measured by ELISA, affinity HPLC and enzymatic method, respectively. On day 29, oral glucose tolerance test (OGTT) (2 g/kg glucose) was carried out under 16-h fasted condition.
**Results:** In vehicle treated db/db mice, during 6 weeks to 10 weeks of age, plasma glucose and HbA1 c were elevated from 238 mg/dl to 625 mg/dl and from 2.7% to 6.3%, respectively. Plasma insulin level was decreased from 37.4 ng/ml to 15.2 ng/ml. In KTO treated group, elevation of plasma glucose and HbA1 c were suppressed. In fact, plasma glucose and HbA1 c changed from 252 mg/dl to 403 mg/dl and from 2.7% to 4.9%, respectively. On day 29, under 16-h fasting condition, plasma glucose level of KTO group was lowered significantly against that of vehicle group (KTO group was 277 mg/dl against vehicle group was 514 mg/dl). But plasma insulin level was not significantly different between these groups at this time. And KTO ameliorated impaired glucose tolerance, at 2 h after oral glucose challenge the plasma glucose of KTO group was 280 mg/dl against that of vehicle group was 569 mg/dl. In KTO treated group, decline of plasma insulin was suppressed. The plasma insulin of KTO group remained its high level and slightly changed from 37.3 ng/ml to 39.4 ng/ml.
**Conclusion:** Because beta3-AR agonist suppressed the decline of plasma insulin level, we conclude that beta3-AR agonist have a protective effect against destruction of pancreatic beta-cells, and that is one of the mechanisms that beta3-AR agonist reduce plasma glucose in db/db mice.

# PS 57

# Insulin secretagogues: clinical studies

## 694

**Tolerability and safety profiles of metformin-glibenclamide combination tablets: new analyses from multicentre, parallel-group, double-blind, randomised, clinical trials**

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**Background and aims:** Hypoglycaemia is an important tolerability issue with insulin secretagogues, including when used in combination therapies. A single-tablet combination of metformin and glibenclamide (Glucovance®) is widely available for use in type 2 diabetes. New analyses from well-designed clinical evaluations of this treatment have been performed to clarify the nature of hypoglycaemic symptoms associated with this treatment.
**Materials and methods:** Data from double-blind, randomised trials in patients hyperglycaemic despite diet and exercise (N=468 and 806), metformin (N=411) or a sulphonylurea (N=639) were pooled for post-diet and exercise and post-monotherapy patients.
**Results:** While hypoglycaemic symptoms with combination tablets were more frequent than with glibenclamide alone, biochemically-confirmed hypoglycaemia (FPG <2.8 mmol/L [50 mg/dL]), treatment discontinuations for hypoglycaemia, and the incidence of hypoglycaemic symptoms rated by investigators as 'severe', were low and similar for each (table). Fewer patients withdrew for hyperglycaemia on combination tablet therapy, consistent with the greater efficacy of this treatment vs. monotherapy. Withdrawals for adverse events (AE) unrelated to glycaemia and serious AE were infrequent in all groups.
**Conclusions:** Combination tablets were well tolerated with reference to biochemically-confirmed or severe hypoglycaemic symptoms, withdrawals for this reason, or serious AE. Most hypoglycaemic symptoms with combination tablets were not accompanied by blood glucose values within the hypoglycaemic range. Such symptoms may be associated with larger falls in blood glucose observed with combination tablets compared with glibenclamide alone. Earlier absorption of glibenclamide from combination tablets, compared with glibenclamide alone, has been observed. As combination tablets are taken with meals, a greater part of the post-treatment insulin response may coincide with the postprandial glucose surge, compared with glibenclamide monotherapy. This may limit the risk of biochemically-documented hypoglycaemia, while supporting efficacy against postprandial hyperglycaemia.

**Incidence (% patients) of selected adverse events (AE) and withdrawals for AE pooled from double-blind, randomised trials in patients randomised to metformin-glibenclamide combination tablets (M-G), metformin (M) or glibenclamide (G)**

| Previous treatment: | Diet and exercise | | | Oral monotherapy | | |
|---|---|---|---|---|---|---|
| | M-G (n=328) | M (n=323) | G (n=311) | M-G (n=261) | M (n=257) | G (n=267) |
| Hypoglycaemia symptoms (%) | 35.4 | 10.5 | 29.9 | 10.0 | 0.8 | 4.1 |
| Confirmed hypoglycaemia | 8.2 | 0.3 | 8.4 | Not evaluated | | |
| Withdrew for hypoglycaemia | 2.1 | 0.0 | 2.3 | 0.4 | 0.0 | 0.0 |
| Withdrew for hyperglycaemia | 3.0 | 9.0 | 8.7 | 1.5 | 11.7 | 7.1 |
| Withdrew for other AE | 4.0 | 5.6 | 3.5 | 3.4 | 4.3 | 2.6 |
| Serious AE | 6.2 | 5.0 | 4.8 | 4.0 | 3.1 | 4.1 |

Data for the dose of M-G appropriate for initiating therapy in each patient population shown (250/1.25 mg for post-diet and 500/2.5 mg for post-monotherapy). Metformin treatment was based on 500 mg tablets, glibenclamide on 2.5 mg tablets (post-diet) or 5 mg tablets (post-monotherapy). Dosages were optimised for therapeutic response, except for patients previously receiving a sulphonylurea who received glibenclamide 20 mg/day throughout.

*The studies included in these analyses were supported by grants from the Bristol-Myers Squibb Company or Merck Sante.*

## 695

**Effect of metformin vs. repaglinide on glycaemic control and non-glycaemic cardiovascular risk-factors in non-obese patients with Type 2 diabetes mellitus (T2DM) uncontrolled by diet (The ReMet-study)**

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**Background and aims:** Metformin is the drug of first choice in obese patients with type 2 diabetes (T2DM) due to its antiglycaemic as well as its cardiovascular protective potentials. However, in non-obese T2DM patients insulin secretagogues are emperically considered the drug of first choice. The aim of the study was to evaluate the effect of Metformin vs an insulin-secretagogue, Repaglinide on glycaemic control and non-gycaemic cardiovascular risk-factors in non-obese patients with T2DM.
**Materials and methods:** Single-center, double-blind, randomized, 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 hypoglycaemic agents. After a one month run-in on diet-only treatment mean $HbA_{1c}$ (SE) was 8.1 (0.1) %, and patients were randomized to either Repaglinide (Rep) 2 mg × 3 followed by Metformin (Met) 1g × 2 or vice versa each for a period of four months with a one month wash-out between interventions. Primary end-point was $HbA_{1c}$.
**Results:** The mean (SE) $HbA_{1c}$ level was significantly lower during Rep vs Met treatment (7.6 (0.1) vs 7.9 (0.1)%, p=0.03). The change in fasting blood glucose was 2.5 (0.3) mmol/l in both groups. Fasting plasma insulin and c-peptide as well as body-weight was significantly higher during Rep vs Met treatment (insulin: 43 (2) vs 36 (3) pmol/l, p=0.001; c-peptide: 752 (26) vs 647 (27) pmol/l, p=0.0003 and body-weight: 76.2 (0.3) vs 73.6 (0.3) kg, p<0.0001). Urinary-albumin-excretion-rate (UAE) and heart-rate (HR) were sligthly lower during Rep vs Met treatment (mean (95%CI): UAE: (5 (2–11) vs 6 (3–15) mg/d, p=0.01, HR: 75 (72–78) vs 77 (74–80) b/min, p=0.01). 24-hour blood-pressure, free-fatty-acids, triglycerides, total-, HDL- and LDL-cholesterol levels were similar during the two treatment-periods.
**Conclusions:** Repaglinide has a superior effect on glycaemic control compared with Metformin in non-obese patients with T2DM. Since Metformin was not associated with any superior beneficial effect on conventional cardiovascular risk-factors as compared with Repaglinide treatment, our results supports the current practice of insulin-secretagogues (e. g. Repaglinide) being the first drug of choice in non-obese patients with T2DM.

## 696

**An alternative treatment with glibomet in obese Type 2 diabetic patients on insulin therapy**

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**Background and aims:** Obese Type 2 diabetic patients are often on abusive insulin treatment. Aim of the study was to evaluate the efficacy of Glibomet (Glibenclamide 2,5 mg and Metformin 400 mg) treatment in such patients after suppression of insulin therapy for 8 weeks.
**Materials and methods:** 50 obese Type 2 diabetic patients (32 females, 18 males); BMI 32 ± 3 kg/m$^2$; diabetes duration 12,5 ± 4,5 years; $HbA_1c$ 11,6 ± 2,8%; insulin dose 94 ± 16 IU/day. All patients were on insulin therapy. Study protocol: duration 8 weeks; all patients discontinued insulin treatment and were administered a diet of 20 kcal/kg of ideal body weight (56% carbohydrates, 34% proteins, 22% lipids with similar amounts of mono-, poly- and saturated fats, fibre 24g/1000 kcal). Patients were randomly assigned to 3 treatments: A) Glibomet 3 times/day (18 patients); B) Glibenclamide 5 mg 3 times/day (14 patients); C) Metformin 850 mg 3 times/day (18 patients). Biochemical and hematological routine, lactic acid, insulin and glucose values both fasting and 2 h postprandial, $HbA_1c$ and ECG were measured before and after treatment.
**Results:** Significant reduction was achieved for fasting glucose (–48%), 2 h postprandial glucose (–42%), body weight (–17%), $HbA_1c$ (–29%), fasting IRI (–62%), 2 h postprandial IRI (–23%), total cholesterol (–26%), triglycerides (–37%) only in group A. No hypoglycemias or side effects were recorded in all groups.
**Conclusion:** Data support the efficacy and safety of Glibomet, but not of glibenclamide or metformine alone, associated with reduced caloric intake in obese Type 2 diabetic patients on previous abusive insulin therapy.

## 697

**Postprandial hyperglycaemia induces platelet activation in Type 2 diabetes mellitus: effects of treatment with repaglinide and glibenclamide**

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**Background and aims:** Type 2 diabetes mellitus is associated with platelet hyperreactivity, endothelial dysfunction and an increased risk of cardiovascular complications. Postprandial hyperglycaemia, an early defect in type 2 diabetes, may induce platelet activation and could contribute to cardiovascular complications. The aim of this open randomized cross-over study was to assess the effects of treatment with repaglinide compared to glibenclamide on platelet and endothelial function in patients with mild type 2 diabetes mellitus, without macrovascular complications, before and after a standardized meal.
**Materials and methods:** Fifteen patients with type 2 diabetes mellitus (age 53 ± 6 years; BMI 28 ± 4 kg/m$^2$; HbA1 c 6.8 ± 1.7%) were investigated on three occasions: at baseline, without antidiabetic treatment, and after 6 weeks treatment with repaglinide or glibenclamide, respectively. Platelet and endothelial function were measured by ADP-induced P-selectin expression using flow cytometry in whole blood, and plasma soluble P-selectin and von Willebrand factor with immunoassays, before and after a standardized meal. Repaglinide or glibenclamide were administered directly before the meal. Blood was drawn for analysis of glucose and insulin premeal and every 10 min until 1.5 h after the meal.
**Results:** Metabolic control (HbA1 c and lipids) did not differ during treatment with repaglinide or glibenclamide. Fasting glucose and incremental peak glucose during the meal were reduced during both treatments compared to baseline (p<0.05 for all; Repeated measures ANOVA; post hoc Student´s t-test for paired data) and P-insulin levels increased during the meal (p<0.01 for both). Postprandial hyperglycaemia increased ADP-induced platelet P-selectin expression in type 2 diabetic patients at baseline as well as after treatment with repaglinide and glibenclamide (p<0.01 for all; Repeated measures, ANOVA). Treatment with repaglinide reduced the overall ADP-induced P-selectin expression (p=0.009), compared to baseline, but the treatment effect was not related to postprandial hyperglycaemia (p=0.32). No inhibiting effect on platelet reactivity was found after glibenclamide treatment compared to baseline, but there was no significant difference between the two antidiabetic treatments. Soluble P-selectin was not affected by postprandial hyperglycaemia and the levels did not differ between treatment groups. Von Willebrand factor levels before the meal were reduced during treatment with both repaglinide and glibenclamide (p<0.05; Repeated measures, ANOVA, post hoc Student´s t-test for paired data), but were not altered postprandially.
**Conclusion:** Premeal administration of repaglinide and glibenclamide has equal efficacy in controlling postprandial hyperglycaemia. Premeal treatment with repaglinide and glibenclamide does not affect platelet reactivity induced by postprandial hyperglycaemia in patients with mild type 2 diabetes mellitus, when metabolic control is kept unaltered. Thus, potential beneficial long-term treatment effects of repaglinide and glibenclamide on platelet and endothelial function seem to be related to other factors than controlling postprandial hyperglycaemia.
*Supported by: Novo Nordisk*

## 698

**Repaglinide treatment does not suppress adiponectin levels despite elevated insulin secretion in Type 2 diabetes patients**

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**Background and aims:** Adiponectin (ADN) is an adipose-specific protein which has been shown to improve insulin action and is also suggested to exert antiatherogenic effects. A short-term post-prandial regulation of ADN concentrations was observed in obese individuals. Insulin decreased ADN concentration in euglycemic clamp study in non-diabetic subjects. Our aim was to study a possible relationship between drug-stimulated insulin secretion and control of the ADN secretion in obese individuals with type 2 diabetes.
**Materials and methods:** This single-blind randomised three period crossover study was conducted in 21 patients with type 2 diabetes mellitus (13 m/8f; 57 ± 8.8 years; BMI 29.7 ± 3.4 kg/m$^2$; mean ± S.E). After two week wash-out period (metformin 2000 mg/d) they received additionally

repaglinide (1 mg tree time daily) or placebo for one week. In the second week patients were treated in cross-over. Liquid meal challenge tests (Biosorb® Energie, Pfrimmer Nutricia, Germany; 36.8 g carbohydrate, 11.6g fat, 12g protein, 300 kcal per 200 ml) with single pre-prandial doses of REP or PL were performed at the end of each treatment period. Adiponectin and insulin were measured in the venous blood samples basal and at 120, 240 min after meal challenge. Wilcoxon-test for paired data was used for statistical comparisons.
**Results:** Basal and postprandial ADN concentrations were not different between both treatments (p=0.93 for basal state, p=0.87 for 120 min, p=0.38 for 240 min). Basal insulin and blood glucose concentrations were not significantly different between both treatments. Post- challenge insulin concentrations at 120 and 240 min were higher with repaglinide compared to placebo (p=0.033 and p=0.015, respectively).
**Conclusion:** Short-term treatment with repaglinide has no effect on basal and post-prandial ADN concentrations despite a significant increase in insulin secretion in patients with type 2 diabetes. Thus, the elevated insulin secretion does not suppress adiponectin levels, indicating that short-acting insulin secretagogue repaglinide does not unfavourably affect this antiatherogenic protein in obese type 2 diabetes patients.

## 699

**Acute and chronic effects of nateglinide on postprandial hypertriglyceridemia induced by a high-fat diet in Goto-Kakizaki rats**
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**Background and aims:** Postprandial hypertriglyceridemia is a characteristic pathophysiological abnormality of type 2 diabetes that has been reported to be an important factor in the development of vascular complications.We previously reported that nateglinide (NT) restores early-phase insulin secretion, especially portal insulin levels, in obese and non-obese diabetic rats and suppresses the increase of plasma triglycerides (TG) including chylomicrons and VLDL after an oral fat emulsion load. In the present study, we examined the effect of NT on postprandial hypertriglyceridemia induced by a high-fat diet in Goto-Kakizaki (GK) rats, and the long-term effect of suppressing postprandial hypertriglyceridemia by NT on hepatic expression of genes involved in lipogenesis and lipid oxidation.
**Materials and methods:** A high-fat diet containing 30% beef tallow was provided to GK rats (9 wks old) twice a day in the dark period (9 AM to 10 AM and 3 PM to 4 PM), and either the vehicle (0.5% methylcellulose) or NT (50 mg/kg) was administered by oral gavage just before each meal twice daily for 12 weeks.
**Results:** Delayed insulin secretion, showing a peak at 3 hrs after meals, occurred with consumption of the high-fat diet. Along with delayed insulin secretion, plasma TG were markedly elevated at 2 hrs postprandially (329 ± 84 mg/dl at 2 hrs vs 147 ± 54 at baseline, $p<0.05$). The same plasma TG pattern was also observed after the 2nd meal each day. In addition, the plasma free fatty acid level also fluctuated widely during consumption of the high-fat diet. NT restored early-phase insulin secretion (1 hr after meal, NT: 14.6 ± 2.6 ng/ml vs vehicle: 5.4 ± 1.6, $p<0.05$) and significantly suppressed the postprandial increase of plasma TG ($\Delta TG_{[0\text{-}2\ hr]}$, NT: 33 ± 13 mg/dl vs vehicle: 181 ± 36, $p<0.05$). The decrease of TG caused by NT was mainly due to a decrease of chylomicrons and VLDL subfractions, as shown by agarose gel electrophoresis. NT also suppressed the variation of free fatty acid levels. Consumption of the high-fat diet by untreated GK rats for 12 wks resulted in an increase of body weight, epididymal fat pad weight, fasting TG, fasting blood glucose, liver weight, and the liver TG content compared with the values in GK rats fed a normal diet. NT treatment significantly suppressed the increase of liver weight (untreated GK: 10.9 ± 0.2 g vs NT: 10.0 ± 0.3, $p<0.05$) and there was a tendency to decrease liver TG content by NT treatment (untreated GK: 241 ± 25 mg/g vs NT: 215 ± 13), although the other parameters were not affected. The increase of liver weight was strongly correlated with the increase of the liver TG content. Hepatic expression of lipogenic genes, such as SREBP1 c, fatty acid synthase, and ATP citrate lyase, was up-regulated about 1.5- to 2-fold in untreated GK rats compared with GK rats fed a normal diet ($p<0.05$). NT treatment did not induce further up-regulation of lipogenic mRNA, but significantly up-regulated PPARα and its downstream enzymes, such as acyl-CoA oxidase and acyl-CoA synthetase, by about 1.3- to 2-fold above the levels in untreated GK rats ($p<0.05$).
**Conclusion:** These results indicate that NT might suppress both elevation of exogenous TG and dietary induction of hepatic lipogenesis in the postprandial state. These acute effects of NT might lead to chronic suppression of TG accumulation in the liver through enhancement of lipid oxidation. Therefore, NT treatment may be beneficial for controlling postprandial lipid metabolism and preventing the onset of metabolic syndrome in patients with type 2 diabetes.

## 700

**Effects of gliclazide on platelet aggregation and plasminogen activator inhibitor Type 1 level in Type 2 diabetic patients**
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**Background and aims:** Vascular complications are a common factor determining morbidity and mortality in the diabetic population. In vitro studies have shown that gliclazide has antiplatelet activities. To assess this clinically, we measured the effects of gliclazide on platelet activities and abnormal fibrinolysis in type 2 diabetic patients.
**Materials and methods:** We studied 14 patients aged 38 to 72 years, included 9 men and 5 women with type 2 DM in our hospital for about six months. We switched 2.5 mg of glibenclamide to 40 mg of gliclazide. We modified the dose of gliclazide to keep glycemic control. We measured 10 mM serotonin-induced or 0.5 mM ADP-induced platelet aggregate formation by particle counting using light scattering. We assayed plasma plasminogen activator inhibitor type 1 (PAI-1) using a latex photometric immunoassay system (LPIA) as fibrinolysis inhibition marker. We measured prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (Fbg), thrombin-antithrombin III complex (TAT), plasmin-alpha2-plasmin inhibitor complex (PIC) as coagulation test. Further, we analyzed FPG, HbA1 c, IRI, total cholesterol (T-Chol), triglyceride (TG).
**Results:** After switching to gliclazide, platelet aggregate formation induced by serotonin was significantly reduced ($P < 0.05$, compared to glibenclamide treatment), though T-Chol, TG, PT, APTT, Fbg were not changed. In HbA1 c decreased group (n=5), ADP-induced platelet aggregate formation and plasma PAI-1 level were significantly reduced ($P < 0.05$, compared to HbA1 c increased group, n=9). Multiple regression analysis showed that percent change of ADP-induced platelet aggregate formation ($r = 0.540$, $P < 0.05$) was independently associated with percent change of plasma PAI-1 level in addition to percent change of HbA1 c ($r = 0.657$, $P < 0.05$) ($R = 0.939$, $P < 0.05$), after switching to gliclazide. The other independent varients inclusive of final dose gliclazide, HOMA-R, percent change of PT, percent change of APTT, percent change of T-Chol were not significantly associated with percent change of plasma PAI-1 level.
**Conclusion:** These results indicate that gliclazide inhibit platelet aggregation via the serotonin pathway, independent glycemic control. Furthermore, in the patients whose glycemic control were improved, gliclazide could inhibit ADP-induced platelet aggregation and PAI-I level. Taken together, gliclazide seems to be more useful for diabetic vascular complications.

# PS 58

# Oral agents and islet cell function

## 701

**The impaired acute insulin response in newly diagnosed Type 2 diabetic patients can be restored by nateglinide and repaglinide**

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**Background and aims:** Nateglinide and repaglinide can significantly increase insulin secretion, especially to restore the acute insulin response (AIR) in type 2 diabetic patients. We design this trial to assess modification of insulin secretion in subjects with newly diagnosed type 2 diabetes after medication cessation following 12-week treatment of nateglinide or repaglinide.
**Materials and methods:** The study was a 12-week double-blind, randomized, parallel controlled trial. A total of 42 subjects (14 male, 28 female) with newly diagnosed type 2 diabetes without previous treatment of hypoglycaemic agents were recruited and had the following characteristics: age, 54.7 ± 10.7years; WHR, 0.92 ± 0.05; BMI, 24.2 ± 3.5 kg/m$^2$; fasting plasma glucose (FPG), 9.18 ± 1.71 mmol/L; and HbA1 c, 8.1 ± 1.5%. Patients whose FPG fluctuated less than 2 mmol/L during a 2-week washout with placebos and diet were enrolled. They were randomized into two groups, one group receiving 60~90 mg nateglinide while the other receiving 0.5~1.0 mg repaglinide 10 min before each meal. Before treatment, FPG, HbA1 c and fasting insulin concentrations were measured and intravenous glucose tolerance test (IVGTT) was performed, insulin levels at 2 min, 4 min, 6 min and 10 min after intravenous injection of 50 ml of 50%GS were also measured. After 12 weeks follow up, the above profiles and IVGTT were repeated 24~72 h after medication cessation. FPG, HbA1 c, AIR and area under curve (AUC) of insulin were compared between groups as well as data before and after treatment.
**Results:** 37 subjects had completed the trial, 19 in repaglinide and 18 in nateglinide. There were no statistical differences in gender, age, duration and baseline FPG, HbA1 c, WHR and BMI between the two groups. There were significant reductions in FPG and HbA1 c in both groups. Comparing to baseline, nateglinide had reduced HbAlc by 1.4 ± 1.2% (P<0.001) and FPG by 1.2 ± 1.8 mmol/L (P<0.05), while repaglinide reduced HbAlc by 2.2 ± 1.5% (P<0.001) and FPG by 2.3 ± 1.5 mmol/L (P<0.001). Repaglinide was more effective than nateglinide in reductions in FPG (P<0.05), but there were no statistical differences in HbAlc between groups (P>0.05). A greater increment of AUC of insulin was evidenced in repaglinide than nateglinide (1009 ± 472pM/min vs. 265 ± 554pM/min, P< 0.001). Before treatment, all subjects were loss of AIR, however, after the 12-week trial, some patients restored AIR in a certain degree. AIR in repaglinide was improved after treatment (700 ± 688pM/min after vs. −30 ± 288pM/min before treatment, P< 0.001), so as in nateglinide (327 ± 515pM/min vs. −61 ± 199pM/min, P=0.001), but no statistical differences were seen between groups (P=0.071).
**Conclusion:** Our data support that both repaglinide and nateglinide can significantly lower plasma glucose level and improve insulin secretion. After 12 weeks treatment, some patients can partly restore acute insulin response, and repaglinide seems to be more effective than nateglinide.

## 702

**Two-year effects of pioglitazone addition to sulphonylurea or metformin therapy on 3-Hour OGTT investigations in patients with Type 2 diabetes**

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**Background and aims:** Patients with Type 2 diabetes experience excessive increases in plasma glucose post-meal compared with normal subjects. As the return of plasma glucose concentrations is often so delayed that patients spend around two-thirds of the day in the postprandial state, postprandial glucose levels are more reflective of their overall metabolic state than fasting levels. Furthermore, post-load hyperglycaemia has been found to be a strong determinant of macrovascular disease. In clinical trials, postprandial glucose excursions are commonly approximated using oral glucose tolerance testing (OGTT) as was the case with these 2-year analyses comparing the effects of pioglitazone with metformin or gliclazide during a 3-hour OGTT.
**Materials and methods:** Two 2-year studies, involving 1269 patients with Type 2 diabetes assessed the effects of the maximum tolerated dose of pioglitazone (15–45 mg/day) as add-on therapy to a pre-existing regimen of either sulphonylurea (SU plus pioglitazone versus SU plus metformin) or metformin (metformin plus pioglitazone versus metformin plus gliclazide) at ≥50% of maximal dose or at the maximum tolerated dose. Following a 3-month, forced-titration period, doses of the add-on therapies were maintained at the maximum tolerated dose for the remainder of the two-year studies. At selected centres, blood samples were drawn before and 30, 60, 90, 120 and 180 minutes after ingestion of 75 g of glucose. Area under the curve (AUC) was calculated using the trapezoidal rule for plasma glucose and insulin measured during the OGTT. The incremental AUC (ie increase in glucose/insulin over the fasting value) was calculated for each patient.
**Results:** After 2 years of treatment, pioglitazone reduced glucose excursion whether added to SU or metformin. Neither gliclazide nor metformin resulted in decreases in glucose excursion when added to existing therapy, despite causing increases in post-load insulin. In contrast, pioglitazone achieved decreases in glucose without increasing post-load insulin.
**Conclusion:** These data suggest that pioglitazone may exert a greater effect than metformin in reducing peripheral insulin resistance, whereas metformin may primarily exert its effects by affecting hepatic glucose production. Furthermore, the marked advantage of pioglitazone over both metformin and gliclazide in terms of post-load glucose and insulin excursions may provide important clinical benefits due to the association of post-load hyperglycaemia with cardiovascular outcome.

Mean change in incremental AUC of plasma glucose and insulin in the OGTT

| | Pioglitazone add-on to SU | Metformin add-on to SU | Pioglitazone add-on to metformin | Gliclazide add-on to metformin |
|---|---|---|---|---|
| Mean change in incremental AUC of glucose (mmol*h/L) | −2.2 | 0.1 | −3.6 | 1.4 |
| p-value (between-group comparison) | 0.003 | | <0.001 | |
| Mean change in incremental AUC of insulin (µIU*h/mL) | 1.7 | 4.9 | −4.6 | 5.6 |
| p-value (between-group comparison) | NS | | NS | |

## 703

**Effect of a 6 month thiazolidinedione treatment on intact proinsulin and adiponectin in patients with Type 2 diabetes mellitus**

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**Background and aims:** Elevated fasting intact proinsulin (iPi) and suppressed adiponectin (Ad) have both been described to be suitable indirect laboratory markers for insulin resistance (IR). It is, therefore, to be expected that successful treatment of IR will lead to reduction of iPi and an increase of Ad concentrations. This prospective randomised parallel study was performed to evaluate the effect of pioglitazone and glimiperide on both markers in patients with type 2 diabetes.
**Materials and methods:** In total, 87 patients (33 women, 50 men, age (Mean±SD): 62 ± 9 years, disease duration 7 ± 7 years, HbA1 c: 7.4 ± 0.8%) were included. No significant baseline differences were seen between both treatment groups with regard to HbA1 c, iPi and Ad. The patients either received treatment with pioglitazone (45 mg + optional sulfonylurea) or glimiperide (1–6 mg, + optional metformin in an attempt to optimize the therapy)
**Results:** HbA1 c improved significantly and equally in both treatment groups within six months (Endpoint: P: 6.9 ± 1.0%, G: 6.8 ± 0.6%, n.s.). Insulin resistance as assessed by HOMA score improved with pioglitazone (−45%) and remained stable with glimiperide (−6%, p<0.001 vs. pioglitazone group). Since both, Ad and iPi are known to be influenced by glucose control, the results were adjusted for the HbA1 c-values. A six month treatment with the pioglitazone regimen resulted in a decrease of iPi by 41% (from 18.1 ± 9.5 pmol/l to 11.6 ± 7.8 pmol/l, p<0.001) and an Ad increase by 187% (from 6.4 ± 3.4 µg/ml to 18.4 ± 10.3 µg/ml, p<0.001), while the glimiperide regimen only resulted in a slight decrease of iPi by 10% (from 17.9 ± 12.3 pmol/l to 16.1 ± 9.3 pmol/l, n.s.) and a further slight decrease of Ad by 7% (from 6.4 ± 4.1 µg/ml to 6.0 ± 3.1 µg/ml, n.s.).

**Conclusion:** The PPARγ agonist regimen had a significantly better effect on later stage β-cell dysfunction as assessed by intact proinsulin ($p<0.05$) and hormonal adipose tissue regulation as assessed by adiponectine ($p<0.001$) than the secretagogue regimen. Since both markers have been associated with cardiovascular risk, these results may support the suggestion that independent from glycemic control, pioglitazone has a substantial cardio-protective effect that may not be seen with glimiperide.
*Supported by: Takeda, Germany*

## 704

**The effect of pioglitazone on fasting and postprandial insulin and insulin precursor species in Type 2 diabetes**
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**Background and aims:** Type 2 diabetes is associated with insulin resistance (IR) and increases in fasting serum concentrations of proinsulin (PI) and 32–33 split proinsulin (SPI). Pioglitazone (PIO), a PPARgamma agonist, sensitises tissues to insulin (INS). The aim of this study was to compare the effect of PIO and glibenclamide (GL) on fasting and postprandial INS, PI and SPI in patients with similar glycaemic control.
**Materials and methods:** Twenty-one type 2 diabetic patients were studied in a double-dummy double-blind trial in which patients were randomly allocated to receive either PIO (n=10) or GL (n=11) after 4 weeks without any medication (baseline) during which diet and life-style measures were encouraged. Normal subjects (CONT; n=10) were also studied who received no medication. At baseline all subjects received a test meal and blood was sampled for the following 8 h. Fasting plasma glucose, INS, PI and SPI were measured and IR estimated by the HOMA model. Postprandial glycaemia, INS, PI & SPI were calculated as the area-under-the-curve (AUC). After 20 weeks treatment, patients underwent a repeat study.
**Results:** The results are shown in Table 1. There were no differences in HbA1 c between the PIO and GL groups but fasting glucose concentrations were lower in the PIO group. At baseline, fasting INS, PI and SPI were greater in the type 2 diabetic patients than CONT. PIO treatment reduced fasting INS, PI and SPI content to levels that did not differ significantly from CONT whilst GL treatment caused an increase in INS with a non-significant trend for PI and SPI levels to increase. HOMA-IR was significantly greater in the type 2 diabetic patients at baseline than in CONT but while GL treatment caused an increase, PIO caused a decrease but remained greater than CONT. Postprandially, GL augmented the AUC of INS, PI and SPI whereas PIO reduced the AUC for PI and SPI, but not INS, to levels not significantly different from CONT. (The data shown in Table 1 are mean ± SEM; * significantly greater than control, $p<0.05$ ANOVA; ‡ significantly greater than baseline (paired t-test) $p<0.05$, † significantly lower than baseline (paired t-test) $p<0.05$)
**Conclusion:** PIO treatment decreases fasting and postprandial insulin precursors in type 2 diabetes compared to standard therapy at similar glycaemic control.

Table 1: The effect of pioglitazone on fasting and postprandial INS, PI and SPI

| | CONT | GL - baseline | GL - treated | PIO - baseline | PIO - treated |
|---|---|---|---|---|---|
| HbA1c (%) | - | 7.38 ± 0.28 | 7.29 ± 0.18 | 7.21 ± 0.19 | 7.20 ± 0.40 |
| HOMA-IR | 1.5 ± 0.1 | 4 ± 0.5* | 6.0 ± 0.8*‡ | 5.1 ± 1.2* | 3.0 ± 0.5*† |
| Fasting glucose (mmol/L) | 5.30 ± 0.30 | 9.39 ± 0.61* | 9.84 ± 0.54* | 9.69 ± 0.57* | 7.36 ± 0.46*† |
| Fasting INS (pmol/L) | 43 ± 4 | 71 ± 9* | 94 ± 13*‡ | 80 ± 17* | 58 ± 10† |
| Fasting PI (pmol/L) | 3 ± 0.5 | 12 ± 2* | 19 ± 5* | 9 ± 2* | 7 ± 2† |
| Fasting SPI (pmol/L) | 4 ± 0.2 | 17 ± 2* | 20 ± 4* | 16 ± 4* | 10 ± 4† |
| AUC glucose (mmol/L/8h) | 40 ± 2 | 66 ± 4* | 76 ± 5* | 77 ± 4* | 62 ± 4*† |
| AUC INS (pmol/L/8h) | 707 ± 60 | 1430 ± 143* | 1909 ± 223*‡ | 1284 ± 175* | 1033 ± 140* |
| AUC PI (pmol/L/8h) | 51 ± 7 | 280 ± 47* | 346 ± 31* | 169 ± 46* | 117 ± 30† |
| AUC SPI (pmol/L/8h) | 80 ± 9 | 345 ± 44* | 380 ± 57* | 251 ± 52* | 181 ± 49† |

## 705

**Plasma C-reactive protein lowering effect of rosiglitazone associated with improving glycemic control**
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**Background:** Highly sensitive C-reactive protein (hs-CRP) is a newly identified risk factor for cardiovascular disease, associated with insulin resistance. Thiazolidinedione is one of a new class of anti-diabetic drugs which improves insulin sensitivity by altering adipocyte metabolism, and, which has potential for prevention of cardiovascular disease. How thiazolidinedione affects plasma hs-CRP is unknown, but it may be due to improve insulin sensitivity, increase plasma adiponectin, alter plasma lipoprotein and/or improve glycemic control.
**Aim:** To study effect of rosiglitazone on plasma highly sensitive C-reactive protein (hs-CRP) in type 2 diabetics.
**Materials and methods:** A group of 13 type 2 diabetics (7M/6F, 46 ± 2 years) from Srinagarind Hospital were enrolled voluntarily after giving informed consent. None was on any insulin-sensitivity altering medication. Before and 4 months after treatment with rosiglitazone 4 mg a day, insulin sensitivity and total body composition were measured using the 120-minute euglycemic hyperinsulinemic clamp (40 $mU \cdot min^{-1} \cdot m^{-2}$ body surface area) and Dual Energy X-ray Absorbtiometry (DEXA). Plasma adiponectin and hs-CRP were measured before and after treatment. Data are presented as means ± SE. Before and after treatment results were compared using the paired t test.
**Results:** Four months of rosiglitazone significantly improved glycemic control, HbA1 c (8.6 ± 0.5 Before vs. 6.7 ± 0.3% After, $p < 0.001$) and insulin stimulated glucose uptake (3.9 ± 0.4 vs. 6.2 ± 0.7 $mg \cdot kg^{-1}$-FFM$\cdot min^{-1}$, $p < 0.001$). Rosiglitazone increased both fat mass (19.1 ± 1.5 vs. 20.2 ± 1.6 kg, $p < 0.05$) and plasma adiponectin (3.6 ± 0.8 vs. 8.2 ± 1.6 $\mu g \cdot mL^{-1}$, $p < 0.001$). Rosiglitazone decreased plasma hs-CRP by a half (2.2 ± 0.5 vs 1.1 ± 0.3 $mg.mL^{-1}$, $p<0.05$). Changes in plasma hs-CRP closely associated with changes in glycemic control (HbA1 c $r = 0.7$, $p < 0.05$ and FPG $r = 0.7$, $p < 0.05$) but did not associate with changes in insulin stimulated glucose uptake, LDL-cholesterol, HDL-cholesterol, triglyceride, fat mass and plasma adiponectin.
**Conclusion:** Rosiglitazone treatment decreased plasma hs-CRP in patients with type 2 diabetes. Plasma hs-CRP lowering effect of rosiglitazone associated with improving glycemic control but did not associate with changing in insulin sensitivity, plasma lipoprotein or plasma adiponectin.
*Supported by: The Endocrine Society of Thailand*

## 706

**Thiazolidinediones improve beta-cell function in Type 2 diabetic patients**
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**Background and aims:** Thiazolidinediones (TZDs) improve glycemic control and insulin sensitivity in patients with type 2 diabetes mellitus (T2DM). There is growing evidence in animal and in vitro studies showing that TZDs improve pancreatic beta cell (beta-cell) function. The aim of this study was to determine whether the TZD-induced improvement in glycemic control is associated with improvement in pancreatic beta-cell function.
**Materials and methods:** 30 type 2 diabetic patients (age=53 ± 2 yr; BMI = 29.4 ± 0.8 $kg/m^2$; fasting plasma glucose [FPG] =10.3 ± 0.4 mM; HbA1 c = 8.2 ± 0.3%) were randomized to 4-months of treatment with a thiazolidinedione (TZD): pioglitazone (PIO, n=9), rosiglitazone (ROSI, n=10) or placebo (Plc, n-10). All subjects received a 75g OGTT with determination of glucose, insulin and C-peptide conc every 15 min for 2 hours. Before and after TZD treatment, insulin secretion was evaluated by deconvolution of C-peptide data and insulin sensitivity by the 2-step euglycemic insulin (40 and 160 $mU \times m^{-2} \times min^{-1}$) clamp with [3-$^3$H]glucose.
**Results:** TZD improved fasting plasma glucose (ΔFPG = −1.3 ± 0.4 [PIO] and −2.8 ± 0.6 [ROSI] vs 0.8 ± 0.4 [Plc] mmol/l), mean plasma glucose during the OGTT (ΔAUC-glucose = −0.20 ± 0.08 [PIO], −0.43 ± 0.07 [ROSI] vs 0.11 ± 0.08 [Plc] mol/l), insulin-mediated total-body glucose disposal (ΔTGD step 1, 8.3 ± 3.2 [PIO] and 1.7 ± 1.8 [ROSI] vs 0.1 ± 1.3 [Plc] and ΔTGD step2, 15.7 ± 3.9 [PIO] and 15.2 ± 4.0 [ROSI] vs −3.8 ± 3.1 [Plc] $\mu mol \times kg^{-1}$ fat-free mass $\times min^{-1}$) and decreased mean plasma FFA during the OGTT (ΔAUC-FFA −8.0 ± 6.9 [PIO] and −16.8 ± 4.7 [ROSI] vs 11.0 ± 7.5

[Plc] mEq/l) (all $p < 0.01$, by ANOVA). The insulin secretory response to the glucose load was significantly improved in both TZD-treated groups (ΔAUC ISR/ΔAUC-glucose + 13.8 ± 5.8 [PIO] + 12.1 ± 4.8 [ROSI] vs – 1.8 ± 4.1 [Plc] nmol/l, $p<0.04$ Plc vs TZD) and this increase was correlated with the improvement in TGD as measured during 160 mU insulin clamp ($r=0.39$, $p<0.03$) and inversely to the improved suppression of FFA during OGTT ($r=0.41$, $p<0.03$).
**Conclusion:** in type 2 diabetic patients, TZD treatment induces recovery of pancreatic beta cell function, probably mediated by the reduction in plasma FFA and FFA metabolities within the beta cell.

# PS 59

# Type 2 diabetes: animal-based studies

## 707

**Different effects between beta-3 adrenergic (AR) agonist and PPAR gamma agonist on adipose tissue in obese (fa/fa) Zucker rats**

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**Background and aims:** To clarify the difference of effects of AR beta-3 agonist (KTO-7924; KTO) and PPAR gamma agonist (pioglitazone; PIO) on white adipose tissue (WAT) and brown adipose tissue (BAT), we investigated cellularity after chronic oral administration.
**Materials and methods:** Male obese (fa/fa) Zucker rats and their lean litter rats (13 weeks of age) allowed free access to chow. Rats were orally given KTO twice a day at a dose of 10 mg/kg, or PIO once a day at a dose of 2 mg/kg for 28 days. The blood was withdrawn and retroperitoneal WAT and interscapular BAT were removed for microscopic observation and weighed.
**Results:** Chronic administration to rats with these agonist, normalized glycemia, decreased plasma trigyceride and free fatty acid concentration, improved insulin responsiveness. OGTT was improved in both groups compared with vehicle group. Body weights (BW) of AR beta-3 agonist, KTO group were decreased, otherwise the BW of PPAR gamma agonist, PIO group were increased compared with vehicle group. Food intake in both groups was not different from the vehicle group. Mean WAT weights in KTO group was decreased to 22 ± 2 g, otherwise the weights in PIO group was increased to 39 ± 2 g compared with 30 ± 2 g in vehicle group. Mean BAT weights in KTO group was increased to 3.1 ± 0.2 g and the weight in PIO group was increased to 4.5 ± 0.2 g compared with 2.2 ± 0.2 g in vehicle group. We examined the cellularity. Mean cell area of WAT was decreased significantly to 7442 ± 169 $\mu m^2$ in KTO group, and increased significantly to 8491 ± 158 $\mu m^2$ in PIO group compared with 7928 ± 153 $\mu m^2$ in vehicle group. Mean oil droplet area of BAT was decreased significantly to 58 ± 2 $\mu m^2$ in KTO group, and decreased significantly to 320 ± 10 $\mu m^2$ in PIO group compared with 365 ± 8 $\mu m^2$ in vehicle group.
**Conclusion:** These data shows that both AR beta-3 agonist and PPAR gamma agonist showed the amelioration of insulin resistance, however, the effects on adipose tissue were clearly different between AR beta-3 agonist and PPAR gamma agonist.

## 708

**Novel anti-diabetic compound BLX-1002 with no affinity to PPARα, γ and δ, lowers serum triglyceride levels and systolic blood pressure in fructose fed rats**

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**Background and aims:** BLX-1002 is an amino acid conjugated small molecule (MW<500) that has a distinct mode of action as compared to any known anti-diabetic compound. In insulin resistant *db/db* mouse model, at a dose of 12.5 mg/kg bodyweight (daily single oral dose) it was able to reduce blood glucose levels more than 40% within 4–5 days of treatment. BLX-1002 lowers serum triglycerides, free fatty acids and total cholesterol levels compared to vehicle treated animals. It does not show any body weight gain compared to control group. It improves the oral glucose tolerance in high fat diet model in C57 mice. When orally given, it has strong inhibitory effect on lipopolysacharide (LPS) induced serum TNF- (72%↓) and IL-6 (42%↓) cytokines production in C57 mice compared to controls.
**Materials and methods:** To find out the affinity of BLX-1002 to PPARα, γ and δ, transactivation studies were carried out in NIH 3T3 cells transiently transfected with the full length or chimeric respective PPAR genes and the reporter construct. Rosiglitazone, pioglitazone and WY-14643 were kept as respective controls for different assays. BLX-1002 did not show any activity in these experiments with any of the PPARs.
Insulin resistance, hyperinsulinemia, hypertriglyceridemia, and hypertension developed in Sprague-Dawley (SD) rats fed a fructose-enriched diet - a cluster of abnormalities seen in patients with Metabolic Syndrome. Given the importance of Metabolic Syndrome as a risk factor for coronary heart disease, attenuation of these defects would be of great use. For this, SD rats were fed a fructose-enriched diet (60%) for 30 days with the addition of BLX-1002 (50 mg/Kg/day) or vehicle for the last 15 days.

**Results:** Both groups gained weight at the same rate, but fructose-fed rats treated with BLX-1002 had significantly lower mean serum triglycerides (270 vs 440 mg/dl, $p < 0.05$) concentrations, as well as lower systolic blood pressure (131 vs 156 mm Hg, $p < 0.05$) at the end. It also lowers serum insulin and free fatty acid levels compared to non treated animals.
In toxicological studies, BLX-1002 did not show any major toxicological effects even at 200 mg/kg in rats and at 150 mg/kg in dogs treated for 28 days.
**Conclusion:** These results suggest that BLX-1002 is a novel, orally active small anti-diabetic compound and can be useful for the insulin resistance and associated complications. Its potential as a candidate for the treatment of Type-II diabetes is currently being evaluated in Clinical studies.

## 709

**Lack of correlation between antihyperglycaemic effect of metformin or rosiglitazone and plasma or tissue-associated dipeptidyl peptidase-IV activity in *db/db* mice**

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**Background and aims:** The glucose lowering effect of metformin and PPARγ agonists has been hypothesised to be, in part, due to increased incretin activity via inhibition and/or decreased secretion of dipeptidyl peptidase-IV (DPP-IV), an enzyme involved in rapid *in vivo* degradation of incretin peptides. Since metformin (Met) and PPARγ agonists act via different mechanisms, the above hypothesis raises the possibility that improvements in glycaemia *per se* may suppress DPP-IV activity and hence impact incretin action.
**Materials and methods:** In *db/db* mice, we determined the effect of chronic administration of Met or the insulin sensitising PPARγ agonist rosiglitazone (RSG) on DPP-IV activity in plasma and several tissues that show prominent DPP-IV activity namely liver, kidney, duodenum and colon. The same parameters were evaluated following administration of Compound BI-B, a potent and selective inhibitor ($IC_{50}$ *vs* human DPP-IV~1nM), with antihyperglycaemic activity.
**Results:** Treatment of *db/db* mice for 3 months with Met (300 mg/kg/day), RSG (3 mg/kg/day) suppressed fasting glucose levels by 5 mM in both cases ($p<0.001$ vs. vehicle). At the end of this chronic dosing regime, plasma DPP-IV activity was unchanged by Met but was increased by 230% with RSG ($p<0.001$ vs. control), despite their equivalent glycaemic control. Met had no effect on DPP-IV activity associated with duodenum or colon but caused a significant ($p<0.05$) increase in DPP-IV activity in liver (+20%) and kidney (+34%). RSG had no effect on DPP-IV activity associated with these organs. In a separate study, the DPP-IV inhibitor Compound BI-B administered to *db/db* mice (1 mg/kg/day), improved fasting hyperglycaemia by 4.6 mM, 6 weeks post-treatment. This was associated with suppression of DPP-IV activity in plasma (−75%, $p<0.001$), liver (−57%, $p<0.001$), kidney (−56%, $p<0.001$) and duodenum (−63%, $p<0.001$), but no effect was observed in colon.
**Conclusion:** The divergent actions of Met and RSG on plasma and tissue-associated DPP-IV activity despite equivalent glycaemic control, suggests that glycaemia *per se* does not affect the DPP-IV/incretin pathway and that, in *db/db* mice, the effect of Met or RSG on glycaemic index is unlikely to be mediated by the incretin system.

## 710

**CKD-501, a newly synthesized thiazolidinedione, improves glucose metabolism in epididymal adipocytes of Zucker rats**

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**Background and aims:** Oral hypoglycemic sulfonylureas, biguanides and thiazolidinediones have shown the wide range of therapeutic effects and have been therefore widely used. But their various toxicities and side effects have been reported. From this reason many scientists have tried to develop new hypoglycemic agents, which are less or nontoxic and more effective. A newly synthesized CKD-501,a thiazolidinedione derivative, was found to have potential hypoglycemic effect. The present study was aimed to investigate the hypoglycemic mechanism of CKD-501 in severely insulin resistant Zucker rats.
**Materials and methods:** To elucidate the cellular mechanisms of the hypoglycemic action, CKD-501(1,3,10 mg/Kg) was orally administered to genetically obese (fa/fa) Zucker rats for three weeks and insulin binding, 2-deoxyglucose (2-DOG) uptake, translocation of glucose transporters and some postreceptor events were determined in the adipocytes isolated from the rats. This study has been carried out along the „Principles of laboratory animal care" (NIH Publication no. 85-23, revised 1985).
**Results:** Three weeks' oral administration of CKD-501 to the rats resulted in marked reduction of plasma glucose levels (26%) and increased specific [$^{125}$I]insulin binding to the adipocytes isolated from the rats. Both basal and insulin stimulated 2-DOG uptakes were confirmed to be increased in the isolated adipocytes by 52% and 70% respectively. Translocation of glucose transporters (GLUT1 and GLUT4), glucose oxidation (basal 227%, insulin stimulated 71%) and lipid synthesis (basal 719%, insulin stimulated 47%) were also significantly enhanced in the adipocytes isolated from the Zucker rats orally treated with CKD-501.
**Conclusion:** The obtained results suggest that the abnormal glucose metabolism associated with insulin resistance might be improved in the Zucker rats by the stimulation of insulin action with CKD-501.

## 711

**Effect of pioglitazone on visceral adipose tissue nuclear factor-kB from Zucker fatty rats**

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**Background and aims:** Chronic inflammation seems to be linked to obesity, cardiovascular disease, insulin resistance and type 2 diabetes mellitus (DM2). Improving insulin resistance may reduce the development of endothelial dysfunction, the first step of the atherosclerotic process. Thiazolidinediones reduces insulin resistance and may help to reduce endothelial dysfunction and inflammatory response in DM2. Aim: to know whether thiazolidinediones improve endothelial dysfunction and inflammatory response in insulin resistance in Zucker fatty rats
**Materials and methods:** Rats were treated with pioglitazone (3 mg/kg/d) for 12 weeks (n=10) or placebo (n=10). Measurements: NF-kB activation in visceral adipose tissue (EMSA); VCAM-1 protein content in aorta (Western blot); plasma insulin (RIA) and plasma TNF-α (ELISA).
**Results:** Compared to placebo, pioglitazone produced a significant decrease ($p<0.05$) of: 1) NF-kB activation (4.41 ± 0.12 vs 5.00 ± 0.16 A.U., mean±SE); 2) VCAM-1 content (3.68 ± 0.13 vs 4.06 ± 0.06, A.U. mean±SE); 3) plasma insulin (3.4 ± 0.4 vs 16.8 ± 2.2 ng/ml) and 4) plasma TNF-α (14 ± 3 vs 64 ± 30 ng/ml).
**Conclusion:** Pioglitazone decreases inflammation activity in visceral adipose tissue and vascular marker of endothelial dysfunction.
*Grant: FIS*

# PS 60

# Metabolic effects of glitazone therapy

## 712

**Increase in adiponectin levels during pioglitazone therapy is independent from glucose control and from ghrelin levels**

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**Background and aims:** Glitazones have been shown to increase the secretion of the adipocyte-derived hormone adiponectin. On the other hand, the gastric hormone ghrelin is known to suppress adiponectin expression in adipocyte cell culture models. It is a matter of discussion, whether the increase in adiponectin during pioglitazone therapy might be due to a suppression of ghrelin levels.
**Materials and methods:** In 10 patients (age 71 ± 9 years, body-mass-index 29.9 ± 3.6 kg/m$^2$, HbA1 c 6.9 ± 0.5%) with type 2 diabetes and mixed hyperlipoproteinemia, who were already treated with sulfonylureas, we additionally initiated a pioglitazone therapy (30 mg/d) for 12 weeks. To investigate the pioglitazone effect independently from blood glucose, HbA1 c was kept unchanged by reducing the daily dose of sulfonylurea if necessary. Ghrelin concentration (radioimmunoassay, Phoenix Pharmaceuticals, Mountain View, CA, USA) as well as adiponectin levels (ELISA, Biovendor, Heidelberg, Germany) were measured with commercially available kits before and after pioglitazone therapy.
**Results:** As requested blood glucose levels remained unchanged within the 12-week pioglitazone therapy (HbA1 c 6.9 ± 0.5% before vs. 6.9 ± 0.6% after pioglitazone) while body weight increased from 85.1 ± 8.4 to 86.6 ± 8.5 kg ($p<0.05$). Adiponectin concentration increased in all patients from 7.70 ± 2.47 to 23.33 ± 8.28 µg/mL ($p<0.01$). However, low ghrelin concentrations at baseline (323.2 ± 139.9 pg/ml) did not change during therapy (322.8 ± 113.7 pg/ml after 12 weeks; n.s.). No correlations were observed neither between ghrelin and adiponectin nor between body weight and hormones.
**Conclusion:** HbA1 c as well as ghrelin levels were unchanged during pioglitazone therapy. Therefore, the increase in adiponectin levels during pioglitazone was independent from blood glucose control and from ghrelin levels.

## 713

**Effects of addition of rosiglitatzone to combination therapy with insulin and metformin on liver fat, insulin sensitivity, glycemic control and insulin requirements in patients with Type 2 diabetes requiring high doses of insulin**

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**Background and aims:** Recent studies have shown that rosiglitazone, in contrast to metformin, markedly reduces liver fat content in humans. Liver fat content is directly proportional to hepatic insulin resistance and insulin requirements in type 2 diabetes. Addition of rosiglitazone to insulin might therefore ameliorate insulin resistance, reduce insulin requirements and improve glycemic control in patients with high insulin requirements and high liver fat content.
**Materials and methods:** We therefore searched for patients who had had a stable and high (>150 IU/day) insulin dose in addition to 2 gr of metformin for at least 2 years. Insulin sensitivity (euglycemic insulin clamp combined with [3-$^3$H]-glucose for measurement of glucose kinetics) and liver fat content (magnetic resonance proton spectroscopy) were determined during insulin and metformin treatment and 8 months after addition of rosiglitazone (8 mg/day). The patients had to be free of any signs of heart failure prior to participation as judged by clinical examination and echocardiography.
**Results:** At baseline, the insulin dose averaged 229 ± 29 IU/day or 2.2 ± 0.3 IU/kg in the patients (age 43 ± 4 yrs, $HbA_{1c}$ 8.9 ± 0.6%, BMI 34.1 ± 1.2 kg/m$^2$, n=6). During rosiglitazone treatment, insulin sensitivity increased markedly by 152% from 1.3 ± 0.3 to 3.2 ± 0.6 mg/kg· min ($p<0.01$) and liver fat content decreased by 58% from 23.3 ± 4.8 to 9.7 ± 4.6% ($p<0.001$). $HbA_{1c}$ decreased from 8.9 ± 0.6 to 7.5 ± 0.5% ($p<0.05$) and the daily insulin dose decreased by 52% to 110 ± 34 IU/day ($p=0.03$). Body weight increased from 105.4 ± 4.6 to 108.3 ± 3.7 kg which is what can be predicted from the improvement in glycemia (2 kg/1% decrease in $HbA_{1c}$).
**Conclusion:** These data are consistent with the idea that reduction in liver fat is a key mechanism underlying the antihyperglycemic efficacy of TZDs. Patients who are poorly controlled on high doses of insulin and metformin because of a fatty liver may benefit from addition of TZD.

## 714

**Effects of PPAR-α/γ therapy on glucose/lipid metabolism, hepatic fat content, and plasma adiponectin concentrations in patients with Type 2 diabetes**

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**Background and aims:** To examine the effects of pioglitazone and fenofibrate as monotherapy and in combination, 14 type 2 diabetic patients (age = 52 ± 4 years, BMI = 30.9 ± 1.5 kg/m$^2$, $HbA_{1c}$ = 9.0 ± 0.7%) received fenofibrate (200 mg/day for 3 months, n=7) or pioglitazone (45 mg/day for 3 months, n=7), followed by the addition of the other agent for 3 months.
**Materials and methods:** Subjects received 75g OGTT, 4 h euglycemic insulin (80 mU/m$^2$-min) clamp with 3-$^3$H-glucose and hepatic fat measurement (MR spectroscopy) at 0, 3, and 6 months.
**Results:** Following pioglitazone monotherapy, fasting plasma glucose (207 ± 23 to 138 ± 9 mg/dl, $p<0.05$), mean plasma glucose during OGTT (305 ± 24 to 238 ± 12 mg/dl, $p<0.05$) and $HbA_{1c}$ (9.0 ± 0.6 to 7.8 ± 0.4%, $p<0.01$) decreased, while plasma adiponectin conc (5.5 ± 0.9 to 13.8 ± 3.5 µg/ml, $p<0.03$) and insulin-stimulated glucose disposal (Rd) (4.3 ± 0.7 to 7.3 ± 0.8 mg/kg-min, $p<0.005$) increased despite increased body weight (Δ=4.1 kg, $p<0.01$). After fenofibrate monotherapy, fasting plasma glucose (199 ± 25 to 197 ± 27 mg/dl), mean plasma glucose during OGTT (273 ± 22 to 287 ± 18 mg/dl), $HbA_{1c}$ (8.8 ± 0.9 to 8.9 ± 0.7%), plasma adiponectin conc (5.5 ± 1.5 to 5.3 ± 1.4 µg/ml) and Rd (5.2 ± 1.0 to 5.5 ± 1.0 mg/kg-min) did not change. Pioglitazone reduced fasting plasma FFA conc (763 ± 100 to 580 ± 41 µM, $p<0.05$), mean plasma FFA conc during OGTT (586 ± 59 to 375 ± 21 µM, $p<0.01$), fasting plasma triglyceride conc (188 ± 25 to 143 ± 20 mg/dl, $p<0.05$) and hepatic fat content (20.4 ± 4.8 to10.2 ± 2.5%, $p<0.02$). Following fenofibrate, fasting plasma FFA conc (718 ± 51 to 812 ± 109 µM), mean plasma FFA conc during OGTT (495 ± 47 to 460 ± 58 µM) and hepatic fat content (19.8 ± 4.0 to 19.0 ± 3.9%) did not change; fasting plasma triglyceride conc decreased from 190 ± 19 to 136 ± 21 mg/dl ($p<0.05$). Addition of fenofibrate to pioglitazone did not change Rd (7.3 ± 0.8 to 7.0 ± 0.8 mg/kg-min), fasting plasma glucose (138 ± 9 to 148 ± 12 mg/dl), $HbA_{1c}$ (7.8 ± 0.4 to 8.0 ± 0.5%), hepatic fat content (10.2 ± 2.5 to 9.0 ± 2.1%) or plasma adiponectin conc (13.8 ± 3.5 to 14.1 ± 4.5 µg/ml); fasting plasma triglyceride conc decreased from 143 ± 20 to 89 ± 13 mg/dl ($p<0.05$). Addition of pioglitazone to fenofibrate increased Rd (5.5 ± 1.0 to 7.1 ± 0.7 mg/kg-min, $p<0.05$) and plasma adiponectin conc (5.3 ± 1.4 to 13.5 ± 3.5 µg/ml, $p<0.01$), while fasting plasma glucose (197 ± 27 to 161 ± 12 mg/dl, $p<0.05$), $HbA_{1c}$ (8.9 ± 0.7 to 8.0 ± 0.5%, $p<0.01$), hepatic fat content (19.0 ± 3.9 to 14.1 ± 2.4%, $p<0.05$) and fasting plasma triglyceride conc (136 ± 21 to 88 ± 10 mg/dl, $p<0.05$) decreased.
**Conclusion:** Combined PPAR-α/γ therapy is associated with a further decrease in plasma triglyceride concentrations compared to PPAR-γ monotherapy, but has no further effect on plasma adiponectin concentrations, hepatic fat content, FFA or glucose metabolism in patients with type 2 diabetes.

## 715

**The effect of dual PPAR α /γ stimulation with combination of rosiglitazone and fenofibrate on metabolic parameters in Type 2 diabetic patients**

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**Background and aims:** Our aim was to assess the additive effect of dual PPAR α /γ induction on insulin resistance and diabetic dyslipidemia which are the main pathogenic factors; independent from blood glucose control, behind macrovascular complications in the setting of type II diabetes. Dual PPAR α /γ stimulation was achieved by addition of fenofibrate (PPAR α agonist) to rosiglitazone therapy (PPAR γ agonist).
**Materials and methods :** 40 type 2 diabetic patients with poor metabolic control (HbA1 c levels between %8.4-%11) who are receiving oral antidia-

betic agents (sulphonylurea, metformin, acarbose) and/or insulin for treatment were included in the study group. Each patient received rosiglitazone 4 mg for 12 weeks. Later fenofibrate 200gm/day was added to therapy regimen for another 12 weeks. Efficacy parameters tested included HbA1 c, uric acid, serum lipid profile and body mass index which were assesed at the start and at 12th and 36th weeks of the study. Safety was assessed by clinical and laboratory (liver and muscle enzymes) monitoring once a month.
**Results:** BMI values at 12th and 36th weeks of the study increased significantly ($p<0.01$) while mean percent reduction for HbA1 c values at 12th and 36th weeks was 11% ($p<0.001$) and 13% ($p<0.002$) respectively. The change in HbA1 c after the additon of fenofibrate to rosiglitazone therapy was not statistically significant ($p<0.1$). The change in LDL levels with roziglitazone at 12th week was not statistically significant ($p<0.1$) while addition of fenofibrate to rosiglitazone decreased mean LDL levels from 126.8 ± 29.6 mg/dl to 106.7 ± 26.7 mg/dl ($p<0.001$). Mean percent reduction for triglyceride levels at 12th and 36th weeks were (19%, 33%, $p<0.001$), and for VLDL levels were (21%, 32%, $p<0.001$). HDL levels increased from 44.59 mg/dl (range 26–68) to 50.14 mg/dl (range 35–72) ($p<0.001$) at 12th week. A further mean increase of %16 ($p<0.001$) was observed after addition of fenofibrate to rosiglitazone. Throughout the study period SGOT, SGPT, GGT, BUN and creatinine values did not significantly changed.
**Conclusion:** In type 2 diabetic patients dual PPAR α /γ stimulation by means of concomitant administration of rosiglitazone and fenofibrate improves the atherogenic dyslipidemic profile in an additive manner with good tolerability.

## 716

**Pioglitazones lowers blood pressure and ameliorates dyslipidaemia in $HbA_{1C}$-responders and non-responders with Type 2-diabetes mellitus**
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**Objectives:** Hypertension (H) and Dyslipidaemia (DYS) are very common comorbidities in patients with type 2-diabetes mellitus (T2DM) and are believed in the majority of cases to be caused primarily by insulin resistance (IR). IR is well documented to play a pivotal role for increased cardiovascular (CV) morbidity and mortality. Pioglitazone (PIO) is a $PPAR_{\gamma}$-agonist which has shown distinct clinical benefits beyond glucose control such as amelioration of H and DYS. Studies confirming these results in large unselected patient populations have not been conducted so far.
**Methods:** 3,785 hypertensive patients with T2DM were recruited and treated with PIO 30 mg od. in combination therapy in an open, multicentre observational trial. Hypertension was classified according to JNC VII. Patients were classified as responders if a reduction in HbA1C of > 0.6% could be observed. Primary parameter was the change in office-based blood pressure measurement, secondary parameter were fasting triglycerides and HDL-cholesterol after 16 weeks of treatment in the intention-to-treat population compared to baseline (BL) by using the LOCF approach. Multivariat analyses for co-variables of treatment response were performed. Patients on lipid-lowering therapies were excluded. Antihyertensive medication was kept stable throughout the observation period. 95% confidence intervals (CI) were calculated.
**Results:** 2,940 (77.7%) and 845 (22.3%) patients were classified as responders and non-responders, respectively.

| Parameter | Mean Adjusted Change to BL [95% CI] Total | Responder | Non-Responder |
|---|---|---|---|
| $HbA_{1C}$ | - 1,34 [± 0,04] | - 1,67 [± 0,04] | - 0,2 [± 0,6] |
| Fasting-Triglycerides (mg/dl) | - 50,1 [± 2,66] | - 55.9 [± 3,11] | - 29.5 [± 4,69] |
| HDL-Cholesterol (mg/dl) | + 3,2 [± 0,83] | + 3.4 [± 0,96] | + 2.7 [± 1,66] |
| Blood Pressure (mmHg) $RR_{syst}$ | - 8,7 [± 0,41] | - 9.3 [± 0,46] | - 6.4 [± 0,86] |
| Blood Pressure (mmHg) $RR_{diast}$ | - 3,8 [± 0,26] | - 4.1 [± 0,30] | - 2.6 [± 0,55] |

BL $HbA_{1C}$, age, diabetes duration or BMI did not correlate with changes of target parameters in either the responder or the non-responder group. In addition, no significant differences for gender as well as metformin or sulfonlyurea co-medication could be observed.
**Conclusions:** PIO reveals significant benefits in ameliorating H and DYS in a sample of unselected hypertensive patients with T2DM. In addition these benefits may also be shown in individuals classically judged as non-responders with regard to glycaemic control. Hence the effects of PIO on H and DYS should be at least only partly dependent on its antihyperglycaemic properties.

## 717

**Muraglitazar, a novel PPAR alpha/gamma dual agonist, lowers fasting plasma glucose, triglycerides, non esterified fatty acids and apolipoprotein (APO) CIII after once a day administration in Type 2 diabetic patients**
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**Introduction:** Muraglitazar is a novel PPAR alpha/gamma dual agonist (non-thiazolidinedione). This oxybenzylglycine analog has been shown to reduce glucose and lipid levels in animal models of diabetes and dyslipidemia.
**Methods:** In this placebo- and active-controlled, multiple ascending dose study, we evaluated the glucose and lipid lowering effects and safety of muraglitazar in type 2 diabetic patients. Subjects with a fasting serum glucose of 150–280 mg/dL were randomized to receive either muraglitazar, placebo, or pioglitazone 45 mg once daily for 28 days while being kept on a standard weight maintaining diet. Muraglitazar dose panels included 1.5 mg, 5 mg, and 20 mg dose groups. Effects on fasting plasma glucose (FPG), Triglycerides (TG), Apolipoprotein (APO) CIII and Non-Esterified Fatty Acids (NEFA) at Day 28 expressed as mean change from pre-treatment baseline are summarized in Table 1.
**Results:** Muraglitazar decreased FPG, TG, APO CIII and NEFA in a dose-dependent fashion. There were no serious adverse events in this study, with muraglitazar being safe and well tolerated following 28 days of treatment up to the 20 mg dose level. In conclusion, muraglitazar treatment exhibits pronounced glucose and lipid lowering effects in patients with type 2 diabetes. These data suggest that muraglitazar has potential as a comprehensive treatment for diabetic patients with and without dyslipidemia.

Table 1: Effects on FPG, TG, APO CIII, NEFA; Mean Change From Pre-Treatment Baseline

| | Placebo N=10 | Pioglitazone 45 mg N=10 | Muraglitazar 1.5 mg N=6 | Muraglitazar 5 mg N=6 | Muraglitazar 20 mg N=6 |
|---|---|---|---|---|---|
| FPG (SD) mg/dL | -54 (61) | -50 (41) | -35 (34) | -101 (91) | -95 (51) |
| % | -24% | -23% | -18% | -34% | -42% |
| TG (SD) mg/dL | -10 (37) | -15 (20) | -9 (64) | -51 (45) | -88 (42) |
| % | -7% | -12% | -2% | -27% | -51% |
| APOCIII (SD) mg/dL | -0.4 (2.7) | 0.1 (2.3) | -0.9 (3.4) | -2 (1.9) | -4 (3.0) |
| % | -4% | +1% | -9% | -16% | -37% |
| NEFA (SD) mmol/L | -0.2 (0.5) | -0.2 (0.2) | -0.3 (0.2) | -0.2 (0.1) | -0.3 (0.2) |
| % | -24% | -23% | -30% | -32% | -54% |

## 718

**Pioglitazone lowers postload glucose and enhances composite insulin sensitivity index during oral glucose tolerance test compared with metformin or gliclazide in patients (pts) with Type 2 diabetes**
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**Background and aims:** Traditionally, oral pharmacological therapy in type 2 diabetes (T2D) has been directed towards improving glycemic control (with or without correcting one or both core defects), insulin resistance and relative insulin deficiency. Pioglitazone (PIO), metformin (MET) and gliclazide (GLIC) lower $HbA_{1c}$ and fasting plasma glucose (FPG) in pts with T2D. As postprandial glucose also influences $HbA_{1c}$ and is a better marker for cardiovascular disease, its importance is now better recognized. We compared the effects of these three drugs, used as monotherapy, on postload glycemia (PG) and composite insulin sensitivity index (CISI) in pts with T2D. CISI is a surrogate for peripheral organ insulin sensitivity.
**Materials and methods:** PG and CISI were analyzed for 623 pts who had an oral glucose tolerance test (OGTT) in two multicenter, randomized, double-blind, parallel group clinical trials (PIO vs MET and PIO vs GLIC). Each study involved a 52-week treatment period consisting of a 12-week (16 for

PIO vs GLIC) forced titration period to maximum tolerated dose of PIO and the active comparator and a subsequent 36- to 40-week maintenance period at this maximum dose. Pts were primarily obese (35–75 yrs; 58% male) with stable or worsening inadequate glycemic control ($HbA_{1c}$ 7.5–11%) for at least three months on diet alone, diagnosed with T2D for four years. $HbA_{1c}$, FPG, plasma glucose and insulin were measured during the 3-hour OGTT done at baseline and after one year of therapy. The change in the incremental area under the curve for glucose ($IAUC_G$) was the surrogate for PG. Insulin sensitivity was evaluated by CISI (10,000/√ [fasting glucose × fasting insulin] × [mean glucose × mean insulin]) using measurements from the first two hours of the OGTT.
**Results:** All therapies lowered $HbA_{1c}$ and FPG when compared with their respective baselines. *PIO vs MET:* Both PIO and MET groups had similar decreases in $HbA_{1c}$ and FPG. Both reduced FSI although only PIO's reduction was different from baseline ($p < 0.0001$) and statistically significantly greater than MET ($p < 0.05$). The PG was reduced more by PIO ($IAUC_G$ = −5.3 mmol*h/l) than by MET (−2.0 mmol*h/l; $p < 0.001$). The change in the incremental area under the curve for insulin ($IAUC_{INS}$) was largely unaffected with PIO (+2.7 µIU*h/ml) compared with an increase with MET (+33.9 µIU*h/ml; $p = 0.049$). PIO and MET both significantly increased CISI vs baseline ($p < 0.001$) with PIO increasing CISI more than MET (1.04 ±.123 vs 0.549 ± 0.125; p = <0.01). *PIO vs GLIC:* PIO and GLIC reduced $HbA_{1c}$ and FPG with no difference between the two groups. PIO significantly decreased whereas GLIC significantly increased FSI from baseline, and the difference between the groups favored PIO ($p < 0.001$). PIO ($IAUC_G$ = −5.0 mmol*h/l) lowered PG more than GLIC (−0.4 mmol*h/l; $p < 0.001$). GLIC increased $IAUC_{INS}$ (30.4 µIU*h/ml; $p < 0.001$), whereas there was no significant change for PIO (+0.3 µIU*h/ml). PIO lowered $AUC_{INS}$ compared with GLIC ($p < 0.001$). PIO also increased CISI vs baseline ($p < 0.001$), whereas GLIC caused no change. The treatment effect favored PIO (1.25 ± 0.117 vs − 0.196 ± 0.126; p< 0.001).
**Conclusions:** PIO improves PG and CISI better than MET or GLIC when used as monotherapy in pts with T2D and may be considered where insulin resistance is a core defect.

## 719

**Effects of pioglitazone versus metformin addition to sulphonylurea therapy and pioglitazone versus gliclazide addition to metformin therapy on HOMA-%S, an estimate of insulin sensitivity, in patients with Type 2 diabetes – 2-year data**

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**Background and aims:** Insulin resistance and β-cell function are the two core defects of Type 2 diabetes. Homeostasis model assessment (HOMA) is an indirect method commonly used in clinical trials to estimate insulin sensitivity (HOMA-%S) and β-cell function (HOMA-%B). This analysis assessed the long-term (2-year) effects of the addition of pioglitazone or gliclazide to metformin and the addition of pioglitazone or metformin to sulphonylurea (SU) in patients with Type 2 diabetes.
**Materials and methods:** In a 2-year study, patients with Type 2 diabetes ($HbA_{1c}$ ≥7.5% and <11%) receiving metformin (at ≥50% maximal or the maximum tolerated dose) were randomised to add-on therapy with either pioglitazone (15 mg/day titrated to 45 mg/day, n=317) or gliclazide (80 mg/day titrated to 320 mg/day, n=313). In a second 2-year study, patients receiving SU at ≥50% maximal or the maximum tolerated dose were randomised to add-on therapy with either pioglitazone (15 mg/day titrated to 45 mg/day, n=319) or metformin (850 mg/day titrated to 2550 mg/day, n=320). HOMA-%S and HOMA-%B were calculated. Following a 3-month, forced-titration period, doses of the add-on therapies were maintained at the maximum tolerated dose for the remainder of the 2-year studies.
**Results:** Insulin sensitivity, as determined by HOMA-%S, was enhanced with the addition of pioglitazone to metformin therapy, but deteriorated when gliclazide was added to metformin (changes from baseline of 11.8% and −5.0%, respectively; p<0.001). When pioglitazone was added to SU there was also an improvement in insulin sensitivity (7.1%) compared with 3.9% when metformin was added to SU (p=NS between groups). There was also a steady and maintained increase in HOMA-%B with pioglitazone and metformin add-on therapies. When gliclazide was added to metformin there was a large rapid increase of approximately 35% in HOMA-%B from baseline to Week 16; however, this was followed by a progressive decline of almost 20% by Week 104. In contrast, HOMA-%B increased by approximately 10% with pioglitazone to Week 24 and was maintained thereafter.
**Conclusion:** Following the initial rapid onset of action, there was a substantial decline in HOMA-%B with gliclazide over time. In contrast, pioglitazone add-on therapy showed sustained improvements in insulin sensitivity and HOMA-%B, which may reflect a lessened burden on already failing β-cells. This may have important implications for the choice of therapy for long-term treatment of Type 2 diabetes mellitus.

## 720

**Comparison of the effectiveness of rosiglitazone and metformin therapy by homeostasis model assessment**

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**Background and aims:** To evaluate the effectivenes of two different insulin sensitizers, rosiglitazone and metformin and to demostrate the factors influencing the clinical efficacy.
**Materials and methods:** One-hundred two poorly controlled type 2 diabetics with sulphonylureas [62 women and 40 men, age 55 ± 9.2 years, body mass index 27.7 ± 1.7 kg/m², and HbA1 c %8.5 ± 0.7 (means±SD)] were randomly assigned into 3 group for the addition of either rosiglitazone or metformin or combination of both and followed up for 1 year. A decrease in HbA1 c level was compared with baseline factors including homeostasis model assessment of insulin sensitivity (HOMA-R) and beta-cell function (HOMA-beta).
**Results:** The overall decrease in HbA1 c levels were similar for the rosiglitazone (−1.1 ± 0.2%) and metformin (−1.2 ± 0.%) groups whereas significantly greater decline in combination group (−1.7 ± 0.4%;p<0.05) was assessed. In the rosiglitazone group, the decrease in HbA1 c levels was negatively correlated with baseline HOMA-R (r =−0.664, p<0.0001) and HOMA-beta (r =−0.634, p<0.0001). In contrast, decrease was positively correlated with baseline HOMA-beta (r=0.567, r=0.465, p<0.0001) both in the metformin and combination group respectively. Multivariate analysis revealed that either HOMA-R OR HOMA-beta was a main determinant of the decrease in HbA1 c levels both in the rosiglitazone and combination group. In the metformin group, baseline levels of fasting glucose were also included as an independent determinant in addition to HOMA-beta, same as in combination group. The subjects with greater HOMA-R (> or=4.0) or HOMA-beta (> or=40%) displayed better response to rosiglitazone or combination therapy than to metformin alone. By week 52, the mean increases in beta-cell function were 37.39% and 42.21% with rosiglitazone and combination, respectively. Furthermore, all of the therapies show that decreases in insulin resistance are sustained for at least 12 months.
**Conclusion:** Addition of rosiglitazone or metformin or combination of them to poorly controlled Type 2 diabetic patients with sulphonylureas, resulted in a comparable reduction in HbA1 c levels. Rosiglitazone is more effective in patients with greater insulin resistance or preserved beta-cell function, whereas metformin is more effective in patients with reduced beta-cell function.

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# Randomised trials in Type 2 diabetes

## 721

**Two-year sustained efficacy of the addition of pioglitazone versus gliclazide addition to metformin therapy in Type 2 diabetes**

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**Background and aims:** This 2-year study compared the efficacy and safety of pioglitazone addition to metformin with gliclazide addition to metformin.
**Materials and methods:** Patients with inadequately controlled Type 2 diabetes ($HbA_{1c}$ ≥7.5 and <11%), despite treatment with metformin at ≥50% maximal or the maximum tolerated dose, were randomised to add-on therapy with pioglitazone (15–45 mg/day, n=317) or gliclazide (80–320 mg/day, n=313). Depending on tolerability, doses of pioglitazone and gliclazide were titrated during the first 16 weeks of the study and maintained thereafter. $HbA_{1c}$ and fasting plasma glucose (FPG) were measured throughout the study.
**Results:** At baseline, mean $HbA_{1c}$ and FPG levels were 8.7% and 11.8 mmol/L, respectively, in the pioglitazone group and 8.5% and 11.3 mmol/L, respectively, in the gliclazide group. Glycaemic parameters showed evidence of better sustainability when pioglitazone was added to metformin compared with gliclazide addition. At Week 104, the $HbA_{1c}$ reduction from baseline was 0.89% when pioglitazone was added to metformin compared with 0.77% for gliclazide addition to metformin (p=NS between groups, ITT population, last observation carried forward). Of particular interest were the patterns of glycaemic control over time. In the gliclazide group, there was an increase in mean $HbA_{1c}$ of 0.70% from the nadir at Week 16 to Week 104. In contrast, the onset of effect was slower with pioglitazone, with the nadir at Week 24, but improvements were better maintained thereafter, with an increase in $HbA_{1c}$ of only 0.22% from Week 24 until Week 104. These between-group differences were even more striking for FPG, with a statistically significantly greater decrease from baseline observed at Week 104 in the pioglitazone group compared with gliclazide (–1.8 mmol/L with pioglitazone versus –1.1 mmol/L with gliclazide; p<0.001). The difference between treatments was also statistically significant for $HbA_{1c}$ in the subgroup of patients who had received at least 72 weeks of treatment (p=0.003; per-protocol population). These differences in the time courses of glycaemia were quantified using the coefficient of failure for $HbA_{1c}$ (0.20%/year for pioglitazone versus 0.44%/year for gliclazide; p<0.001). More than 70% of patients completed the study and the overall safety profile was comparable in terms of the number of patients reporting adverse events. There was a higher incidence of hypoglycaemia with gliclazide and more oedema with pioglitazone.
**Conclusion:** Due to its better sustainability of glycaemic control, as demonstrated over 2 years in this study, the addition of pioglitazone to patients failing metformin therapy may offer a distinct advantage over the addition of sulphonylurea for the long-term treatment of Type 2 diabetes mellitus.

## 722

**Long-term efficacy and safety of pioglitazone in comparison to gliclazide in patients with Type 2 diabetes**

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**Background and aims:** Previous studies have shown that pioglitazone (PIO) exerts anti-hyperglycemic effects in patients with T2DM by improving insulin resistance. However, large-scale, long-term studies comparing the efficacy and safety of PIO with other hypoglycemic drugs in patients with T2DM are scarce. Moreover, insulin-sensitizers, such as pioglitazone (PIO), should affect favourably not only glucose and lipid homeostasis but also coagulation and thrombosis.
**Materials and methods:** In this multicenter, double blind, randomized, two parallel group study, the efficacy and safety of PIO (30–45 mg/day) were compared with GLI (80–320 mg/day) for 12 months in 225 patients with T2DM. Efficacy variables were the changes over a year of HbA1 c, fasting blood glucose (FBG), fasting insulin and C-peptide levels and insulin resistance assessed with HOMA. In a subgroup of patients (n=7) systemic glucose production (SGP) was determined by a combination of isotopic (deuterated glucose) and clamp techniques. Safety variables (hematologic and biochemical parameters) were also assessed.
**Results:** HbA1 c decreased significantly (p<0.01) in both groups (–0.9% and –0.7% in PIO and GLI group, respectively, p<0.01), without any statistical difference between groups. A statistically reduction of FBG was observed at any time point in both groups (p<0.01 vs baseline), but the slope of the reduction after 6 month was significant different between groups (p<0.004). Insulin and C-peptide levels, as well as insulin resistance assessed with HOMA, decreased significantly in the PIO group (p<0.01 vs baseline), but not in the GLI groups with significant differences between groups (p<0.01). Interestingly, SGP but not glucose utilization decreased in the PIO group (p<0.05), but not in the GLI group. A significant decrease of platelets (p<0.05 after 12 months), vWF and PAI-1 (p<0.01 after 6 and 12 months), and an increase of AT-III (p<0.01 after 6 and 12 months) and fibrinogen (p<0.05 after 6 and 12 months) were observed in the PIO group, but not in the GLI group. A statistically decrease of ALT from baseline was observed in the PIO group and in comparison with the GLI group at any time point (p<0.01). Similarly, AST decreased (p<0.01) at a greater extent (p<0.05) after 3 months of PIO treatment. Moreover, γGT and ALP decreased significantly in the PIO group after 6 and 12 months (p<0.01), but not in the GLI group, and the difference between groups was also significant (p<0.01).
**Conclusion:** Glucose control improved similarly in PIO and GLI over a year, but PIO sustained a continuous decrease of fasting blood glucose, whereas GLI failed to maintain a similar trend. In addition, PIO had favourable effects on insulin resistance, and insulin and C peptide levels, and decreased SGP during isoglycemic glucose clamp. In addition, long-term exposure to PIO did not determine any hepatic impairment, whereas improved coagulation parameters independently of blood glucose control. Thus, the combination of effects not only on glucose and lipid homeostasis but also on coagulation and thrombosis is likely to impact favourably on vascular disease in patients with T2DM.

## 723

**Long-term (2-year) effects on serum lipids of pioglitazone, gliclazide and metformin as add-on therapies in patients with Type 2 diabetes**

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**Background and aims:** Diabetic dyslipidaemia is characterised by increased triglycerides (TG) and decreased high-density lipoprotein cholesterol (HDL-C), whereas low-density lipoprotein cholesterol (LDL-C) is often within the normal range or borderline elevated in patients with Type 2 diabetes. Studies of up to one year's duration have shown the beneficial effects of pioglitazone on TG and HDL-C. Two-year analyses presented here compare the effects on lipids of pioglitazone with gliclazide when added to metformin and of pioglitazone addition to sulphonylurea (SU) compared with metformin addition.
**Materials and methods:** Patients with Type 2 diabetes ($HbA_{1c}$ ≥7.5% and <11%), currently receiving metformin at ≥50% maximal or the maximum tolerated dose, were randomised to either pioglitazone (15–45 mg/day; n=317) or gliclazide (80–320 mg/day; n=313) as add-on to metformin in one trial. In the other trial, pioglitazone (15–45 mg/day; n=319) or metformin (850–2550 mg/day; n=320) was added to SU. Doses of study drug were titrated, depending on tolerability, during the first 16 weeks of the studies and maintained thereafter. Endpoints included fasting serum TG, HDL-C, LDL-C and total cholesterol (TC)/HDL-C ratio. Atherogenic index of plasma (AiP), which is inversely correlated with LDL particle size, was calculated using the logarithmic transformation of TG/HDL-C ratio.
**Results:** Baseline lipid values were typical for diabetic dyslipidaemia (2.4–2.8 mmol/L for TG, 1.1 mmol/L for HDL-C, 3.3–3.6 mmol/L for LDL-C and ~5.6 mmol/L for TC). Whether added to SU or metformin, pioglitazone caused significantly greater decreases in TG and increases in HDL-C levels than comparators. These effects were mirrored in the AiP (–0.38 for pioglitazone add-on to SU versus –0.23 for metformin; p<0.001, and –0.46 for pioglitazone add-on to metformin versus –0.14 for gliclazide; p<0.001). Effects on LDL-C were greater with the comparators than with pioglitazone add-on therapy; however, overall lipid profile, as assessed by TC/HDL-C ratio, was improved by a similar amount in the pioglitazone add-on groups and the metformin add-on to SU group, and to a significantly lesser extent by gliclazide add-on to metformin.
**Conclusion:** The superior effects on TG, HDL-C and AiP of add-on therapy with pioglitazone over gliclazide or metformin suggest that pioglitazone may have benefits in terms of targeting specific abnormalities associated with diabetic dyslipidaemia.

Percentage change from baseline lipid values

| | Pioglitazone plus SU | Metformin plus SU | p-value | Pioglitazone plus metformin | Gliclazide plus metformin | p-value |
|---|---|---|---|---|---|---|
| TG | -17% | -9% | =0.001 | -23% | -7% | <0.001 |
| HDL-C | 21% | 15% | <0.001 | 22% | 7% | <0.001 |
| LDL-C | -5% | -11% | <0.001 | 2% | -6% | <0.001 |
| TC/HDL-C ratio | -19% | -17% | =0.282 | -17% | -10% | <0.001 |

## 724

**Evaluation of the effects of rosiglitazone combination therapy on ambulatory blood pressure after 6 months; a 12 month substudy of the RECORD trial in people with Type 2 diabetes mellitus**

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**Background and aims:** RECORD is a multicentre, randomized, open-label, parallel group study investigating the occurrence and progression of cardiovascular disease and glycaemic control in type 2 diabetes mellitus (T2DM) patients. The primary measure of efficacy in this Ambulatory Blood Pressure Monitoring (ABPM) substudy is change from baseline in 24 hour mean diastolic blood pressure (dBP) after 6 months of dual combination therapy.
**Materials and methods:** A total of 759 T2DM patients from the main RECORD study were enrolled into the ABPM substudy. At baseline, patients inadequately controlled on metformin (Met), were randomized to add-on therapy with rosiglitazone (RSG) or sulphonylurea (Su); while patients inadequately controlled on Su, were randomized to add-on therapy with RSG or Met. Analysis was within stratum, based on background therapy upon entry to the study. The analysis of 24 hour ABPM recordings included computation of mean 24 hour, day-time and night-time diastolic and systolic BP. Baseline demography was comparable across treatment groups within stratum and characteristic of a T2DM population. The majority of patients had a diagnosis of hypertension at screening (75%). Modification of blood pressure-lowering medication was permitted at any time. Treatment groups within stratum were comparable in terms of concomitant use of cardiovascular medications.
**Results:** At baseline, 24 h mean diastolic and systolic BP were comparable between treatment groups within stratum. At 6 months, greater reductions from baseline in 24 h mean dBP and sBP were observed in patients treated with RSG combination therapy compared to those treated with the control combinations (see table). Differences between Su+RSG and Su+Met were statistically significant for both diastolic and systolic BP.

| | *Background Met stratum* | | *Background Su stratum* | |
|---|---|---|---|---|
| | Met+RSG | Met+Su | Su+RSG | Su+Met |
| n | 164 | 158 | 155 | 154 |
| | **24 Hour mean Ambulatory dBP at Month 6 (mmHg)** | | | |
| Baseline‡ (± SD) | 77.7 ± 8.3 | 78.1 ± 9.6 | 76.4 ± 7.8 | 75.7 ± 8.1 |
| Month 6‡ (± SD) | 74.5 ± 8.2 | 76.1 ± 8.8 | 73.2 ± 7.7 | 75.4 ± 8.0 |
| Change* (± SE) | -2.9 ± 0.5 | -1.7 ± 0.5 | -3.0 ± 0.5 | -0.3 ± 0.5 |
| Difference from Met/Su* (95% CI) | -1.2 (-2.5, 0.0) (p=0.056) | | -2.8 (-4.1, -1.5) (p<0.0001) | |
| | **24 Hour mean Ambulatory sBP at Month 6 (mmHg)** | | | |
| Baseline‡ (± SD) | 132.1 ± 13.6 | 134.3 ± 15.8 | 131.8 ± 12.8 | 131.9 ± 14.1 |
| Month 6‡ (± SD) | 129.0 ± 12.7 | 132.3 ± 15.1 | 128.8 ± 12.9 | 131.3 ± 13.3 |
| Change* (± SE) | -3.1 ± 0.8 | -1.5 ± 0.8 | -3.4 ± 0.9 | -0.6 ± 0.8 |
| Difference from Met/Su* (95% CI) | -1.6 (-3.7, 0.5) (p=0.126) | | -2.8 (-4.9, -0.6) (p=0.013) | |

‡ Raw means
* Based on ANCOVA with an adjustment for Baseline BP (diastolic or systolic, as appropriate), Age, Gender and Established Hypertension at Screen

**Conclusions:** Adding rosiglitazone to sulphonylurea therapy resulted in clinically and statistically significant reductions in ambulatory BP when compared with sulphonylurea plus metformin combination.
*GlaxoSmithKline, the manufacturer of rosiglitazone, is the sponsor of the ABPM substudy (conducted as part of the RECORD trial).*

## 725

**Rosiglitazone evaluated for cardiac outcomes and regulation of glycaemia in diabetes (RECORD): an interim analysis of glycaemia at 18 months**

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**Background and aims:** RECORD is a multicentre, randomized, open-label, parallel group study of cardiovascular disease and glycaemic control. This interim analysis evaluated glycaemic efficacy after 18 months in two groups of people with Type 2 diabetes mellitus (T2DM). People on metformin (Met) were randomized to add-on therapy with rosiglitazone (RSG) or Sulfonylurea (Su), while people on Su were randomized to add-on therapy with RSG or Met.
**Materials and methods:** Entry criteria were age 40–75 yr, inadequately controlled on maximum permitted or tolerated doses of Met or a Su ($HbA_{1c}$ 7.1–9.0%). Throughout the study patients were treated to target $HbA_{1c} \leq$ 7.0%, being up-titrated to a total daily dose of 8 mg RSG or maximum permitted/tolerated doses of Met/Su. If $HbA_{1c}$ deteriorated to ≥ 8.5%, a third oral glucose-lowering drug was added (RSG-treated group) or insulin started (non RSG group) according to local clinical practice. The interim primary objective was to demonstrate that RSG change from baseline $HbA_{1c}$ at 18 months (ANCOVA, ITT population) was non-inferior to the controls based on a margin of 0.4%. Secondary endpoints included fasting plasma glucose (FPG), HOMA-estimated insulin sensitivity, and C-reactive protein (CRP).
**Results:** At 18 months, there was no significant difference in $HbA_{1c}$ or FPG between RSG and Met or Su add-on therapies in either treatment group (Table). In the completers population, HOMA insulin resistance was substantially reduced in the RSG combination treatment groups compared to the respective control groups (change from baseline Met+RSG 35% vs Met+Su 10%; Su+RSG 40% vs Su+Met 25%). Significant reductions were also observed in CRP (Met+RSG 41% vs Met+Su 6%, p<0.0001); Su+RSG 36% vs Su+Met 16%, p=0.0014).

| | *Background metformin* | | *Background sulfonylurea* | |
|---|---|---|---|---|
| | Met+RSG | Met+Su | Su+RSG | Su+Met |
| n | 252 | 263 | 301 | 271 |
| $HbA_{1c}$ (%) | | | | |
| Baseline (±SE) | 7.85 ± 0.04 | 7.83 ± 0.04 | 7.98 ± 0.04 | 7.97 ± 0.05 |
| Change (±SE) | -0.48 ± 0.06 | -0.55 ± 0.06 | -0.55 ± 0.06 | -0.61 ± 0.05 |
| Difference (95% CI)[a] | 0.07 (-0.09, 0.23) (NS) | | 0.06 (-0.09, 0.20) (NS) | |
| FPG (mmol/l) | | | | |
| Baseline (±SE) | 9.63 ± 0.15 | 9.63 ± 0.14 | 10.13 ± 0.15 | 10.21 ± 0.13 |
| Change (±SE) | -1.53 ± 0.13 | -1.18 ± 0.15 | -1.96 ± 0.15 | -1.62 ± 0.13 |
| Difference (95% CI) | -0.36 (-0.74, 0.02) (p=0.062) | | -0.34 (-0.73, 0.05) (p=0.089) | |

[a], *non-inferiority for RSG set at upper CI <0.40%*

**Conclusions:** In people with T2DM, RSG in combination with Met or Su is as effective in lowering $HbA_{1c}$ as the standard combination of Met+Su, and produces greater improvements in insulin sensitivity and a significant reduction in CRP.
*The RECORD study sponsor is GlaxoSmithKline.*

## 726

**The COMPACT-Study: pioglitazone vs. standard oral antidiabetic agents for treatment of patients with Type-2-diabetes mellitus – focus on metabolic effectiveness**

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**Objectives:** Pioglitazone (PIO), a thiazolidinedione, is a member of a new class of oral antidiabetic agents targeted to treat insulin resistance, the major underlying cause of type-2-diabetes mellitus. Metformin (MET) and Sulfonylurea (SH) represent both the established classes of most commonly used oral antiabetic drugs (OAD). This study is aimed to compare both treatment options in dual combination therapy under the conditions of daily practice with regard to metabolic control.
**Methods:** Prospective, controlled, non-randomized observational study in 51 outpatient diabetic centres where patient selection, allocation to treatment and dose was left at the physicians discretion. Quality standards included a central laboratory and a regular monitoring. Primary parameter was the change of $HbA_{1C}$ compared to baseline ($\Delta$ $HbA_{1C}$) where a difference of < 0.5% between both arms was set for defining non-inferiority. Analyses were adjusted for baseline.
**Results:** 209 patients were treated with PIO 30 mg and 130 patients 118 Metformin and Sulfonylurea; 3 Metformin without Sulfonylurea; 9 Sulfonylurea without Metformin for 24 weeks

| Parameter (Mean ± SD), [95%CI] | PIO | OAD |
|---|---|---|
| $\Delta$ $HbA_{1C}$ (%) | -0.70 (+/- 0.998) [-0,839; -0,566] | -0.53 (+/- 1.003) [-0.702; -0,356] |
| $\Delta$ Fasting Glucose (mg/dl) | -24.5 (+/- 48.48) [-30.20; -18.81] | -19.6 (+/- 50.76) [-26.96; -12.21] |
| $\Delta$ postprandial Glucose (mg/dl) | -40,9↑↑ (+/- 51.01) [-47.88; -33.93] | -21.7↑↑ (+/- 70.12) [-31.26; -12.09] |
| $\Delta$ Fasting Triglycerides (mg/dl) | -57.1↑↑ (+/- 136.07) [-72.95; -41.29] | -20.9↑↑ (+/- 146.92) [-40.95; -0.88] |
| $\Delta$ HDL-Cholesterol (mg/dl) | +4.6↑ (+/- 11.18) [+3.36; +5.76] | +2.2↑ (+/- 8.82) [+0.68; +3.72] |
| $\Delta$ $HbA_{1C}$ (%) Metabolic Syndrome. (ATP III, NCEP) | | |
| yes | - 0.74 (+/- 1.004) [-0.90; -0.58] | - 0.58 (+/- 1.209) [-0.78; -0.38] |
| no | - 0.46 (+/- 1.015) [-0.81; -0.12] | - 0.30 (+/- 1.120) [-0.69; -0.09] |

↑ $p < .05$ ($\Delta$ values PIO vs. OAD)
↑↑ $p < .01$ ($\Delta$ values PIO vs. OAD)

**Conclusions:** PIO proved to be non-inferior to OAD treatment with regard to $HbA_{1C}$ reduction but reveals additional benefits in terms of extended metabolic control. This holds true especially in insulin resistant patients.

## 727

**Greater benefits of rosiglitazone added to submaximal dose of metformin compared to maximizing metformin dose in Type 2 diabetes mellitus patients**

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**Background and aims:** Rosiglitazone (RSG) is often added to maximal metformin (MET) to provide durable glycaemic benefit in type 2 diabetes mellitus (T2DM) patients requiring combination therapy. Increasing MET monotherapy may be associated with increased gastrointestinal (GI) intolerability.
**Materials and methods:** In this 24-week, double-blind trial, the efficacy, tolerability and safety of adding RSG to submaximal MET (1 g/day) was evaluated vs. titrating to the maximal effective dose of MET (2 g/day). T2DM patients on prior therapy (diet/exercise or oral anti-diabetic mono- or combination therapy) were treated with open-label MET (1 g/day) and after a 4–7 week run-in period, were randomized to receive addition of blinded RSG 4 mg (n = 358) or MET 500 mg (n = 351). At week 8, the groups were uptitrated to a total daily dose of 8 mg + 1 g/day (RSG + MET) and 2 g/day (MET).
**Results:** As designed, $HbA_{1c}$ change from baseline at week 24 for RSG + MET was noninferior to maximal MET. However, RSG + MET was superior to MET in improvements in fasting plasma glucose (FPG), insulin and homeostasis model assessment of insulin sensitivity (HOMA-S).

| Parameter | Mean change from baseline (95% CI) at week 24 | | Treatment effect (95% CI) |
|---|---|---|---|
| | RSG + MET | MET | RSG + MET |
| $HbA_{1c}$ (%) | -0.82 (-0.95, -0.69) (n = 322) | -0.63 (-0.74, -0.52) n = 313) | -0.19 (-0.35, -0.03) |
| FPG (mmol/l) | -2.14 (-2.44, -1.83)† (n = 259) | -1.10 (-1.40, -0.81)† (n = 271) | -0.86 (-1.24, -0.48)† |
| Insulin (pmol/l) | -47 (-58, -35)† (n = 260) | -18 (-30, -6) (n = 268) | -26 (-38., -15.)† |
| HOMA-S % change | 46.5 (39.0, 54.4)† (n = 249) | 14.5 (8.6, 20.8)† (n = 262) | 26.7 (17.9, 36.2)† |

†significant, $P < 0.0001$

A greater proportion of patients in the RSG + MET group reached targets of $HbA_{1c} < 7\%$ (54.7% vs. 45.0%) and FPG ≤ 7 mmol/l (47.5% vs. 28.8%). RSG + MET was generally safe, well tolerated and associated with fewer GI side effects than MET (28.5% vs. 39.1%). Withdrawals due to GI events were lower with RSG + MET (3.1%) than MET (6.8%).
**Conclusions:** These data suggest that the addition of RSG to submaximal MET provided significantly greater improvements in insulin resistance and response to glycaemic targets compared to maximal uptitration with MET and is associated with better GI tolerability.

## 728

**The effects of rosiglitazone in poorly-controlled, drug-naive patients with Type 2 diabetes mellitus**

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**Background and aims:** The UK Prospective Diabetes Study (UKPDS) demonstrated that patients on standard monotherapy for type 2 diabetes mellitus (T2DM) progressively lose glucose control within 3 years of diagnosis. The thiazolidinedione (TZD) class of oral anti-diabetic agents shows promise in its ability to provide durable glycaemic control. Some trepidation exists, however, in starting TZDs as first-line therapy in patients who are poorly controlled because of their longer onset of action compared to standard agents. In this randomized, double-blind study, the safety and efficacy of rosiglitazone (RSG) in poorly-controlled ($HbA_{1c} \geq 10\%$), drug-naive T2DM patients was evaluated.
**Materials and methods:** Eligible patients were randomly assigned to either RSG 4 mg od (n = 73) or RSG 8 mg od (n = 73) and assessed over a 24-week period. Baseline $HbA_{1c}$ (mean ± SD) in both groups was similar; 11.45 ± 1.29% (RSG 4 mg) and 11.42 ± 1.27% (RSG 8 mg).
**Results:** Patients that completed 24 weeks of treatment, demonstrated clinically and statistically significant reductions in $HbA_{1c}$ for both the RSG 4 mg (-2.08% [97.5% CI: -2.76, -1.41]) and RSG 8 mg groups (-2.99% [97.5% CI: -3.63, -2.35]). A greater proportion reached the American Diabetes Association goal of $HbA_{1c} < 7\%$ in the RSG 8 mg group (19/51, 37.3%) than in the RSG 4 mg group (8/44, 18.2%). There was a significant reduction from baseline to week 24 in triglycerides in the RSG 8 mg group (-23.3% [95% CI: -32.3, -8.6]) and an observed reduction in the RSG 4 mg group (-11.8% [95% CI: -21.3, 6.9]) as measured by median percent change from baseline. Significant increases in HDL levels (RSG 4 mg, 7.8% [95% CI: 4.7, 15.8]; RSG 8 mg, 8.4% [95% CI: 2.4, 12.2]) as well as a trend towards improvement in both LDL/HDL and total cholesterol/HDL ratios were observed in both treatment groups. Adverse events were similar to those previously reported for RSG.
**Conclusions:** These data suggest that significant glycaemic and lipid benefits were derived with both doses of RSG. Therefore, for drug-naive patients with T2DM with poor glycaemic control, RSG 4 mg and RSG 8 mg are safe and effective first-line therapies.

## 729

**Rosiglitazone added early to sulphonylurea provides superior control versus uptitration of sulphonylurea**

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**Background and aims:** Traditional approaches to oral antidiabetic therapy, in which agents are added sequentially after single agent failure, can leave subjects undertreated for extended periods. Early initiation of combination therapy may provide better and more durable glycaemic control than that achievable through addition of agents following failure of prior therapy.
**Materials and methods:** In 3 double-blind clinical trials, rosiglitazone (RSG) was added to submaximal sulphonylurea (SU) doses. Subjects taking a half-maximal dose of gliclazide (study 145), glibenclamide (study 162) or glipizide (study 135) were randomized to either RSG added to SU or SU monotherapy. In studies 145 and 162, SU monotherapy was titrated to maximal dose 8 weeks after randomization, while both SU and RSG doses were held constant in the combination groups. In study 135, study medication in both arms was adjusted at study visits, based on glucose levels.
**Results:** Early addition of RSG to SU significantly decreased $HbA_{1c}$ and fasting plasma glucose (FPG) levels compared to uptitration of SU alone following 6 months of treatment, with substantially more subjects reaching the American Diabetes Association $HbA_{1c}$ goal < 7%.

| Study: | 135 GLIP | RSG + GLIP | 145 GLIC | RSG + GLIC | 162 GLIB | RSG + GLIB |
|---|---|---|---|---|---|---|
| n | 105 | 113 | 233 | 218 | 154 | 160 |
| $HbA_{1c}$ (%) baseline | 7.6 | 7.7 | 8.6 | 8.5 | 8.0 | 7.9 |
| Mean Δ from baseline | 0.0 | −0.6* | 0.1 | −1.2* | −0.1 | −0.9* |
| Comparison to SU | – | −0.6* | – | −1.3* | – | −0.8* |
| % with $HbA_{1c} < 7\%$ | 42 | 64 | 22 | 48 | 25 | 57 |
| n | 104 | 106 | 241 | 225 | 170 | 165 |
| FPG (mmol/l) baseline | 8.84 | 8.71 | 10.22 | 10.27 | 9.60 | 9.38 |
| Mean Δ from baseline | 0.26 | −1.39* | 0.51* | −2.41* | 0.18 | −2.15* |
| Comparison to SU | – | −1.72* | – | −2.99* | – | −2.43* |

*$P < 0.05$
GLIP – glipizide; GLIC – gliclazide; GLIB – glibenclamide

In study 135, glycaemic improvements in the RSG + glipizide group were sustained for the 2-year double-blind treatment period. RSG + SU therapy was generally well tolerated in all studies.
**Conclusions:** These studies highlight the limited dose response of SUs and provide consistent evidence that robust, durable glycaemic reductions are achievable by adding RSG to submaximal SU doses. Early combination therapy provided superior glycaemic control and should be considered before continuing monotherapy at increasing doses.

# PS 62

# Short-acting insulin analogues in Type 2 diabetes

## 730

**Influence of various intensive insulin therapy methods on daily insulin requirement in Type 2 diabetes patients**

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**Background and aims:** Poorly controlled, insulin-treated type 2 diabetes mellitus most often requires an intensifying therapy, which leads to achievement of good metabolic control and allow to determine an actual insulin requirement. Multiple doses insulin injection (MDI), intravenous insulin infusion (IVII) and continuous subcutaneous insulin infusion (CSII) may be applied in the above situation as a hospital short-term intensive insulin therapy (IIT). The aim of the study was to determine an effect of MDI, IVII and CSII on daily insulin requirement in poorly controlled type 2 diabetes patients.
**Materials and methods:** 90 poorly controlled, twice-daily insulin treated type 2 diabetes patients (glycated hemoglobin A1c 9.8+/−1.6%) were enrolled into the study. The patients were randomly assigned to one of three groups depending on treatment modalities: MDI (n=30, mean age 63.1+/−4.2 years, mean blood glucose 13.8+/−3.0 mmol/l, BMI 27.8+/−1.6 kg/m$^2$, duration of diabetes 5.6+/−2.6 years), IVII (n=30, mean age 60.9+/−5.4 years, mean blood glucose 13.5+/−1.5 mmol/l, BMI 28.5+/−1.5 kg/ m$^2$, duration of diabetes 5.8+/−3.2 years) and CSII (n=30, mean age 60.3+/−7.1 years, mean blood glucose 13.1+/−3.3 mmol/l, BMI 28.0+/−2.1 kg/m$^2$, duration of diabetes 6.1+/−2.2 years). IIT was discontinued at achievement near-normoglycemia and twice-daily insulin therapy regimen was restituted. Mean duration of MDI was 6.0 +/−2.0 days, IVII was 5.2+/−1.0 days, and CSII 4.5+/−1.2 days. Daily insulin dosage before and after each IIT method was analyzed.
**Results:** Mean daily blood glucose was one day after IIT discontinuation as follows: for MDI: 9.4 +/−2.5 mmol/l, for IVII: 9.1 +/−2.4 mmol/l and for CSII: 9.3 +/−2.2 mmol/l (NS). The 24-hour insulin requirement in MDI, IVII and CSII groups was: 0.72 +/− 0.19, 0.70 +/−0.15 and 0.81 +/−0.33 IU/kg body weight before and after the IIT 0.83 +/−0.24, 0.74 +/− 0.18 and 0.82 +/−0.33 IU/kg body weight, respectively. Significant increase in 24-hour insulin requirement before and after IIT was noted only in MDI subjects ($p<0.001$). **Conclusion:** CSII and IVII, seem to be a better than MDI intensifying insulin therapy regimen which allow to achieve a good metabolic control in type 2 diabetes patients without 24-hour insulin requirement increase.

## 731

**Effectiveness of prandial insulin therapy for patients with Type 2 diabetes – clinical results of "PHAZIT"**

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**Background and aims:** Providing short-acting insulin at mealtimes is an increasingly common therapy for patients with type 2 diabetes, 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 (HUM) – 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 from 53 German outpatient diabetic centres. Patients with insufficient metabolic control ($HbA_{1c}$ >= 7.0% and <= 12.0%) under combination therapy with oral hypoglycaemic agents (incl. MET) were included in the study. At baseline these patients were switched to a combination of ASP/MET or HUM/MET. 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 costs. Preliminary data from 416 patients with type 2 diabetes are presented.

**Results:** Patients in both groups were comparable regarding age, duration of diabetes, gender and known co-morbidities such as hypertension (see table 1). A significant improvement in metabolic control ($HbA_{1c}$ at baseline: 8.8%) was observed in both groups. The observed change in $HbA_{1c}$ was achieved in patients from the ASP/MET group with 15% less daily insulin dosage than patients in the HUM/MET group ($p<0.05$). Moderate weight loss was observed in both groups. A total of 57.5% of the patients in the ASP/MET group achieved weight loss, 11.1% more patients than in the HUM/MET group. The MET dose in both groups was similar.
**Conclusion:** Prandial insulin therapy in combination with MET for at least 24 weeks proved to be very effective at regulating metabolic control. Patients treated with ASP/MET revealed additional benefits in terms of weight loss, and reduced dosage requirement of insulin. Further analysis of these preliminary results will be completed, in addition to a comparison of cost-effectiveness between treatments.

Tab. 1

| Parameter (mean +/- SD); [95%CI] | ASP/MET (n=229) | HUM/MET (n=187) |
|---|---|---|
| Age (years) | **60.8**$^{*}$ (± 11.4) | **63.0**$^{*}$ (± 10.5) |
| Duration of diabetes (years) | **10.1** (± 6.4) | **10.9** (± 7.5) |
| Hypertension (%) | **69.9** | **73.4** |
| $HbA_{1c}$ at baseline | **8.8%** (± 1.05) | **8.8%** (± 1.10) |
| Reduction in $HbA_{1c}$ (%-points) | **-1.5** (± 1.17) [-1.3; -1.6] | **-1.4** (± 1.28)[-1.2; -1.6] |
| Weight at baseline (kg) | **89.5** (± 19.0) | **91.1** (± 18.1) |
| Change of weight (kg) | **-0.83** (± 2.75) [-0.07; -1.58] | **-1.07** (± 4.57) [-0.26; -1.88] |
| Daily dose of metformin (mg) | **1609** (± 538) | **1572** (± 555) |
| Daily dose of insulin (start of treatment) | **23.2**$^{**}$ U (± 13.1) | **27.1**$^{**}$ U (± 14.3) |
| Daily dose of insulin (12 weeks) | **28.7**$^{*}$ U (± 14.6) | **33.2**$^{*}$ U (± 16.6) |
| SD = standard deviation; 95% CI = 95% interval of confidence | U = units; $^{**}p < 0.01$; $^{*}p < 0.05$ | |

## 732

**Comparison of intensive mixture therapy with a basal-only insulin regimen in insulin-naive patients with Type 2 diabetes after failure of dual oral antihyperglycemic agent therapy**
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**Background and aims:** When oral antihyperglycemic (OA) therapy fails to provide adequate glycemic
control, multiple insulin regimens may be utilized: basal insulin used alone or in combination with prandial insulin, or pre-mixed insulin formulations used in a once- or twice-daily regimen or in a prandial multidose regimen. No consensus exists on the optimal insulin regimen that should be used after OA failure. Therefore, we compared the glycemic effects of two different insulin regimens used in combination with previous OAs: intensive mixture therapy (IMT: Humalog® Mix50™ before breakfast and lunch and Humalog® Mix25™ before supper) or insulin glargine given at bedtime. The pre-mixed insulin formulations used in the IMT regimen differ in the proportion of rapid-acting insulin, whereas insulin glargine is a basal-only regimen with no rapid-acting insulin component.
**Materials and methods:** Dual OA failure was determined when therapy with a combination of at least 2 OAs from different classes for a minimum of 2 months failed to provide adequate glycemic control (HbA1 c 1.2–2.0 times the upper limit of normal). After OA failure criteria were satisfied, 60 insulin-naïve patients with type 2 diabetes were randomized to one of the insulin regimens for 4 months with cross-over to the alternative regimen for an additional 4 months. Glycemic goals were preprandial blood glucose <120 mg/dL (6.7 mmol/L) and 2-hour postprandial blood glucose <180 mg / dL (10.0 mmol/L).
**Results:** Baseline HbA1 c was 9.02 ± 1.24% (mean ± SD) for patients first treated with glargine therapy and 9.40 ± 1.41% for patients first treated with IMT. Insulin dose was optimized by investigators without forced titration. IMT was superior to glargine for both endpoint HbA1 c (7.09 ± 0.74% vs 7.33 ± 0.81%, p=0.0026) and HbA1 c change with therapy (−1.08 ± 1.33% vs −0.70 ± 1.40%, p=0068). Fifty percent of patients receiving IMT and 35% of patients receiving insulin glargine achieved HbA1 c <7% at endpoint. Two-hour postprandial glucose values (for all three meals) and pre-dinner glucose values were significantly less with IMT than with insulin glargine (p=0.0034, 0.0001, 0.0066, and 0.0205). Overall hypoglycemia was infrequent (IMT vs glargine: 4.7 ± 6.4 vs 2.3 ± 3.2 episodes/30 days, p<0.001). No severe hypoglycemia was observed during the study with either therapy.
**Conclusions:** After dual OA failure, multiple daily injections of pre-mixed insulin formulations containing a rapid-acting insulin component resulted in superior overall glycemic control as measured by HbA1 c compared with a basal-only insulin regimen. Through the action of the rapid-acting insulin component, IMT was associated with improved postprandial blood glucose control at each meal; most likely this contributed to lower HbA1 c values in this treatment group. IMT also resulted in a greater proportion of patients achieving HbA1 c <7% compared with the basal-only insulin regimen. Furthermore, intensifying insulin therapy through three daily prandial insulin injections not only improved measures of long-term and mealtime glucose control, but the occurrence of severe hypoglycemic episodes was not increased.

## 733

**Pharmacodynamics and pharmacokinetics of insulin glulisine compared with insulin lispro and regular human insulin in patients with Type 2 diabetes**
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**Background and aims:** Insulin glulisine (GLU) is a new, rapid-acting insulin analogue. The pharmacodynamics and pharmacokinetics of GLU were compared with insulin lispro (IL) and regular human insulin (RHI) in a euglycaemic glucose clamp study.
**Materials and methods:** Patients with Type 2 diabetes (n=24; mean±SD; age 57 ± 8 years; BMI 28.5 ± 3.8 kg/m$^2$; $HbA_{1c}$ <11.5%) were enrolled into a randomized, double-blind, incomplete block design study. Prior to insulin injection, subjects' blood glucose values were adjusted to a target level of 7.0 mmol/L (126 mg/dL) using a variable iv infusion of RHI, until 20 minutes before sc injection of 0.2 IU/kg body weight of either GLU, IL or RHI.
**Results:** Maximum serum insulin concentration (INS-$C_{max}$) and initial insulin exposure (INS-$AUC_{0-2\,h}$) were greater for GLU and IL than for RHI, whereas time to INS-$C_{max}$ (INS-$t_{max}$) was not significantly different for all insulins. In parallel, time to 20% and 80% of GIR-AUC (GIR-$t_{20\%}$; GIR-$t_{80\%}$), representing early glucose disposal and duration of bulk of activity, respectively, were shorter after GLU and IL than after RHI in a head-to-head comparison. However, total glucose disposal of all insulins was comparable. Similarly, intra-subject variability was comparable between treatments.
**Conclusion:** In patients with Type 2 diabetes, who are overweight but not obese, GLU and IL presented with super-imposable time-action profiles, which displayed a more rapid onset and shorter duration of action compared with RHI; therefore, GLU qualifies as a rapid-acting insulin analogue.

| | Total geometric mean | | | Head-to-head comparison. Point estimates (95% CI) | | |
|---|---|---|---|---|---|---|
| | GLU | IL | RHI | GLU/IL | GLU/RHI | IL/RHI |
| INS-$C_{max}$ (μU/mL) | 92 | 81 | 46 | 124 (91, 169) | 216 (177, 264)* | 161 (128, 202)† |
| INS-$t_{max}$ (min)‡ | 83 | 71 | 92 | 11 (−15, 31) | −5 (−28, 20) | −22 (−33, 13) |
| INS-$AUC_{0-2\,h}$ (μU.min/mL) | 7661 | 7622 | 4221 | 106 (80, 141) | 202 (160, 256)* | 169 (132, 217)† |
| GIR-$t_{20\%}$ (min)‡ | 121 | 112 | 194 | 9 (−13, 30) | −64 (−103, −37)* | −74 (−115, −46)† |
| GIR-$t_{80\%}$ (min)‡ | 350 | 332 | 435 | 13 (−50, 75) | −72 (−133, −26)* | −94 (−153, −37)† |
| GIR-$AUC_{0-end}$ (mg/kg)§ | 906 | 1129 | 943 | −51 (−523, 421) | 120 (−370, 609) | 121 (−1167, 1409) |

*GLU vs RHI p <0.05; †IL vs RHI p <0.05; ‡median; §arithmetic mean; CI = confidence interval

*This work was supported by Aventis Pharmaceuticals*

## 734

**Glycaemic control with insulin glulisine versus regular human insulin in a basal-bolus regimen in patients with Type 2 diabetes**
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**Background and aims:** This multicentre, randomized, open-label, parallel study was conducted to compare the safety and efficacy of insulin glulisine (GLU) with regular human insulin (RHI), in combination with NPH insulin (NPH).
**Materials and methods:** Patients with Type 2 diabetes (n=876, mean $HbA_{1c}$ [GHb measured as $HbA_{1c}$ equivalents] 7.55%, mean BMI 34.5 kg/m$^2$)

received GLU/NPH (n=435) or RHI/NPH (n=441) for 26 weeks. Bolus and basal insulin doses were titrated to post- and preprandial blood glucose (BG) targets, respectively, while avoiding hypoglycaemia. NPH was administered twice daily; GLU and RHI were administered at least twice daily before breakfast and dinner (immediate self-mixing with NPH was allowed). If required, based on the clinical judgement of the investigator, more than two injections of GLU or RHI were permitted. Subjects were allowed to continue the same dose of pre-study regimens of oral antidiabetic drug (OAD) therapy (unless hypoglycaemia necessitated a dose change).

**Results:** A greater reduction from baseline to endpoint $HbA_{1c}$ was observed with GLU versus RHI, which was statistically significant (-0.46% vs -0.30%, respectively; p=0.0029). In addition, at endpoint, post-breakfast BG was significantly lower with GLU versus RHI (8.7 mmol/L vs 9.0 mmol/L [156 mg/dL vs 162 mg/dL]; p <0.05), as was post-dinner BG (8.5 mmol/L vs 9.1 mmol/L [154 mg/dL vs 163 mg/dL]; p <0.05).In both the GLU and RHI groups, a similar proportion of patients experienced at least one episode of symptomatic (51.7% vs 53.6%, respectively; p=0.600), nocturnal (21.4% vs 24.5%, respectively; p=0.303) or severe hypoglycaemia (1.4% vs 1.2%, respectively; p=0.645). Symptomatic hypoglycaemia rates were also similar in the GLU and RHI groups (0.95 vs 1.04 events/patient-month, respectively; p=0.186), as were nocturnal hypoglycaemia rates (0.14 vs 0.21events/patient-month, respectively; p=0.109). Severe hypoglycaemia rates were low at 0.0041 events/patient-month for GLU and 0.0037 events/patient-month for RHI, and similar between treatment groups (between-treatment p=0.353). Weight gain was also comparable between groups. At endpoint, total daily insulin dose increased similarly with GLU versus RHI (+9.3 IU vs +11.1 IU; p=0.2427), as did the daily basal insulin dose (+5.7 IU vs +6.0 IU; p=0.7741) and the daily short-acting insulin dose (+3.7 IU vs +5.0 IU; p=0.1756). A similar proportion of patients in both groups mixed their insulins in a syringe just prior to injection (GLU: 74.1%; RHI: 83.1%). The average number of prandial insulin injections per day was also similar in both treatment groups (2.27 with GLU vs 2.24 with RHI). Safety variables were comparable between groups in the 26-week study, and these safety profiles persisted in a 26-week extension (total treatment period 52 weeks; n=709).

**Conclusion:** GLU can provide statistically significantly better overall glycemic and postprandial control versus RHI in patients with Type 2 diabetes who are already relatively well controlled on insulin alone or insulin plus OADs.

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## 735

**Time action profiles of insulin aspart and human regular insulin in middle-aged and elderly subjects with Type 2 diabetes**

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**Background and aims:** The rapid onset of action and short duration of action of insulin aspart (IAsp) would make it suitable for post-prandial administration in elderly subjects with type 2 diabetes (ELD) in whom hypoglycemic episodes are difficult to avoid because of their unpredictable eating habits. The time-action profile of IAsp, however, has never been investigated in the main target population. Therefore, we compared the metabolic activitiy of IAsp and human regular insulin (HI) in ELD and middle-aged (MA) subjects with type 2 diabetes.

**Materials and methods:** Nineteen elderly subjects (70 5 ys (mean SD), BMI 30 4 kg/m, HbA1 c 7.7 1.3%) and 18 middle-aged subjects (53 7 ys, BMI 31 3 kg/m, HbA1 c 8.3 1.0%) were enrolled in this double-blind, cross-over trial and received 0.3 U/kg IAsp and HI sc. at two glucose-clamp experiments (clamp-level 10% below fasting blood glucose concentration, clamp duration 10 h).

**Results:** IAsp had a faster onset of action (AUC0–2 h) and a shorter duration of action (time to late half-maximal action t50%) than HI in either patient population (table, fig.).

**Conclusion:** When compared to MA, there was a tendency towards a shorter duration of action for both IAsp and HI in ELD. If confirmed by clinical trials, post-prandial administration of insulin may become a valuable therapeutic option in elderly diabetic patients.

Elderly Subjects

| | Insulin Aspart | Human Insulin |
|---|---|---|
| GIR max (mg/kg/min) | 4.8 ± 2.6 (1) | 4.3 ± 2.3 |
| t max (min) | 225 ± 125 (2) | 299 ± 87 |
| early t 50% (min) | 87 ± 65 (2) | 139 ± 91 |
| late t 50% (min) | 348 ± 93 (1,3) | 497 ± 85 (3) |
| AUC 0–2 h (mg/kg) | 265 ± 192 (1) | 112 ± 66 |
| | (1) p<0.05 vs. HI | |
| | (2) p<0,1 vs. HI | |
| | (3) p<0,1 vs. MA | |

Middle-aged Subjects

| | Insulin Aspart | Human Insulin |
|---|---|---|
| GIR max (mg/kg/min) | 3,7 ± 1.5 (4) | 3.2 ± 1.8 |
| t max (min) | 208 ± 62 (4) | 389 ± 132 |
| early t 50% (min) | 71 ± 28 (4) | 140 ± 93 |
| late t 50% (min) | 408 ± 90 (4) | 535 ± 62 |
| AUC 0–2 h (mg/kg) | 205 ± 128 (4) | 99 ± 64 |
| | (4) p<0,05 vs. HI | |

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## 736

**Comparison between aspart and regular insulin in combination with metformin in Type 2 diabetic patients**

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**Background and aims:** Type 2 diabetes mellitus is a clinical chronic syndrome tipically characterized both by insulin resistance and insulin secretory impairment. Type 2 diabetic patient presents a total absence of the acute or first-phase insulin response with a consequent increase in plasma glucose levels during the post-absorbitive status. For these reasons, a correct clinical approach is based on the pre-meal administration of regular insulin in patients not well controlled with oral hypoglycemic drugs. But this treatment has some side effects, and it is important apply pharmachological treatments able to reduce daily insulin rate. In insulin-treated diabetic patients, co-administration of metformin significantly reduce HbA1 c and/or daily insulin rate, limiting weight gain induced by insulin. Nevertheless significant clinical data about the association between aspart and metformin are not available at this time. The aim of this study is to evaluate the Aspart analogue efficacy, in comparison with regular insulin, in the management of type 2 diabetic patients in secondary failure.

**Materials and methods:** This study was designed as a randomised, open-label, cross-over clinical trail. The study was performed at Outpatient Clinic of the Section of Metabolic Diseases and Diabetes of the University of Florence. 30 type 2 diabetic patients were enrolled. They had a mean age of 65 ± 3.9 years, a mean Body Mass Index of 27.7 ± 3.81, duration of diabetes was17,5 ± 12,7 years and a residual pancreatic function with c-peptide 0.9 ± 0.6 nmol/L, HbA1C>7.5%. After receiving instructions on how to self-inject insulin doses and self-monitor glycemic levels, patients were randomly assigned to one of two different treatments: A) Human regular insulin 30' before breakfast, lunch, dinner + metformin 500 mg after meals, B) Aspart immediately before breakfast, lunch, dinner + meal metformin 500 mg after meals. After 90 days, patients assigned to treatment A were switched to treatment B and vice-versa for the following 90 days. After 90 and 180 days all patients performed a glycemic meal test evaluating at baseline, 30', 60', 90', 120', 150', 180': glycemia and lipid profile. The meal consisted in 35 gr CHO, 16 gr lipids, 26 gr protein + 2 gr fiber. The primary endpoint of the study was the variation of HbA1C measured at baseline and at the end of each 90 day treatment period. Secondary end points of the study were: lipid profile, glycemia and lipid profile after standard meal and hypoglycaemic events. We calculated the area under curve for all these parameters by Tai's formula.

**Results:** During the study no serious hypoglycemic events were observed in the two groups. Our data showed that, while at the begining of the study, there was not significant difference in HbA1C between patients assegned to aspart and to human regular insulin HbA1C was significantly reduced with Aspart but not with human regular insulin treatment. Mean reduction was 0.35 ± 0.7 vs 0.14 ± 0.7; p=0.04. Glucose AUC after the meal test was significantly lower with Aspart when compared to regular human insulin treat-

ment (p=0.006), while no significant treatment related difference was observed in AUC of lipid parameters.
**Conclusion:** These results, all together, encourage short-term analogue aspart administration in combination with metformin, in type 2 diabetic patients with postprandial hyperglycemia not satisfactorily controlled with only oral hypoglicemic agents.

## 737

**Effects on postprandial metabolism of insulins differing for action rapidity (aspart vs. regular) in patients with Type 2 diabetes**

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**Background and aims:** Type 2 diabetic patients present postprandial lipoprotein abnormalities mainly concerning chylomicrons and large VLDL. Blood glucose levels, and even more, a different insulinization in the postprandial period could influence these abnormalities. The aim of this study was to evaluate how different plasma levels of glucose and insulin, obtained through a more or less rapid prandial insulinization, may influence postprandial lipemia in patients with Type 2 diabetes.
**Materials and methods:** Fifteen patients with Type 2 diabetes in stable blood glucose control with diet or oral hypoglycemic drugs (8 male, 7 female, age 56 ± 7 years, BMI 26 ± 2 kg/m$^2$, fasting plasma cholesterol 180 ± 26 mg/dl and triglycerides 101 ± 53 mg/dl, M±SD) were administered on two different occasions one week apart a standard meal (944 kcal; CHO 31%, fat 57%, protein 12%). Before the meal, on one occasion Regular human insulin and on the other the fast-acting analogue Aspart were administered subcutaneously (0.15 IU/kg body weight). Before the meal and over the 6 following hours blood samples were taken for the determination of glucose, and cholesterol and triglycerides concentrations in whole plasma and in lipoproteins and lipoprotein subfractions (chylomicrons, large VLDL, small VLDL, IDL, LDL, HDL).
**Results:** Blood glucose levels during the early postprandial phase were significantly lower after Aspart ($p<0.05$ vs. Regular at 2 and 3 h), while similar levels between the two insulin types were observed in the later phase. Blood glucose postprandial incremental area (IAUC) was significantly lower after Aspart than after Regular (−261 ± 41 vs. −92 ± 45 mg/dl·6 h, $p<0.05$, M±SEM). Postprandial lipid levels were not substantially different after the two insulin types in whole plasma (triglycerides IAUC 400 ± 49 and 402 ± 61 mg/dl·6 h, cholesterol IAUC -13 ± 12 and −14 ± 11 mg/dl·6 h, after Regular and Aspart, respectively), as well as in chylomicrons, large VLDL and the other lipoproteins.
**Conclusion:** In Type 2 diabetic patients a more rapid insulinization during the postprandial period significantly reduces blood glucose levels, particularly in the early phase, without substantial differences of postprandial lipemic response, at least under acute conditions.

## 738

**Targeting postprandial rather than fasting blood glucose results in better overall glycemic control in patients with Type 2 diabetes**

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**Background and aims:** The objective of this study was to compare the glycemic response to an insulin lispro mixture (25% insulin lispro and 75% neutral protamine lispro [NPL]) twice daily combined with metformin (Mix25+M) to that of once-daily insulin glargine (G) plus M in patients with type 2 diabetes inadequately controlled with once or twice-daily insulin alone, or in combination with oral agents.
**Materials and methods:** Ninety-seven patients were randomized in a multicenter, open-label, 32-week crossover study. During the 6-week lead-in period, all patients were treated with bedtime neutral protamine Hagedorn (NPH) insulin plus M ≥1500 mg/day. During the treatment phase, study insulins were titrated to achieve target premeal blood glucose (BG) of 5–7 mmol/L (Mix25+M and G+M) and 2-h postprandial (pp) BG of 8–10 mmol/L (Mix25+M). Primary variables included hemoglobin A1C (A1C), 2-h pp BG, hypoglycemia rate (episodes/patient/30 days) and incidence (% patients experiencing ≥1 episode) of overall nocturnal hypoglycemia and daily insulin dose.
**Results:** Endpoint A1C was lower with Mix25+M compared to G+M (7.54 ± 0.87% vs. 8.14 ± 1.03%, $p<0.001$). A1C change (baseline to endpoint) was greater with Mix25+M (−1.00% vs. −0.42%; $p<0.001$). 2-h pp BG was approximately 1.5 mM lower ($p<0.001$) after all 3 main meals during treatment with Mix25+M. Fasting BG values were lower with G+M (7.90 ± 1.92 vs. 7.39 ± 1.96 mmol/L; p=0.007). Patients treated with Mix25+M had a lower rate of nocturnal hypoglycemia (0.14 ± 0.49 vs. 0.34 ± 0.85 episodes/patient/30 days; p=0.002), while overall hypoglycemia rate was not different between treatments (0.61 ± 1.41 vs. 0.44 ± 1.07 episodes/patient/30 days; p=0.477). Endpoint insulin dose was higher with Mix25+M (0.42 ± 0.20 vs. 0.36 ± 0.18 U/kg/d; $p<0.001$). Patients treated with Mix25+M experienced slightly more weight gain than those treated with G+M (p=0.001).
**Conclusions:** In patients with type 2 diabetes inadequately controlled on once or twice-daily insulin alone or in combination with oral agents, twice-daily Mix25+M (a treatment primarily targeting pp BG) provided a clinically significant improvement in A1C and reduced nocturnal hypoglycemia at a slightly higher fasting BG and daily insulin dose compared with once-daily G+M, a treatment targeting fasting BG.

# PS 63

# Long-acting insulin analogues in Type 2 diabetes

## 739

**Insulin glargine in Type 2 diabetes: an observational study of everyday practice**

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**Backgrounds and aims:** Many patients with Type 2 diabetes are inadequately controlled with oral antidiabetic (OAD) treatment alone. The aim of this observational study was to investigate the effect of adding insulin glargine (LANTUS®), a long-acting basal insulin analogue, to support OAD treatment in patients with Type 2 diabetes in everyday practice.
**Methods and materials:** In this 9-month, uncontrolled observational study, 12,216 patients with Type 2 diabetes not adequately controlled on OADs received add-on insulin glargine treatment. Dosing decisions, including any changes to OADs, were made at the physicians discretion, reflecting everyday practice. At 9 months, a sub-analysis according to different baseline body mass index (BMI) groups was performed to determine changes in $HbA_{1c}$, fasting blood glucose (FBG) and BMI. These groups were as follows: Group 1: 1891 patients with BMI <25 kg/m$^2$; Group 2: 5355 patients with BMI ≥25 kg/m$^2$ and <30 kg/m$^2$; Group 3: 2789 patients with BMI ≥30 kg/m$^2$ and <35 kg/m$^2$; and Group 4: 1055 patients with BMI ≥35 kg/m$^2$.
**Results:** Prior to the study, 93.47% of patients received OAD mono- or combination therapy. At baseline, mean (± standard deviation [SD]) age was 64 (± 11.3) years. Duration of diabetes was >5 years in 47% of patients, 1–5 years in 39% of patients and <1 year in 10% of patients. The remaining 4% of patients were newly diagnosed. At 9 months, the addition of insulin glargine to OADs led to reductions in $HbA_{1c}$ and FBG in all BMI groups (Table). At endpoint, $HbA_{1c}$ was comparable between treatment groups. The insulin glargine dose increased in each group; however, this was associated with reduced BMI (Groups 2–4), except in Group 1. At baseline and endpoint, patients with a higher BMI (Groups 3–4) were treated with more insulin than patients with a lower BMI (Groups 1-2). For an additional 1126 patients, BMI at baseline was not reported. Of 47 adverse events documented, 19 were due to hypoglycaemia.

| | $HbA_{1c}$ (%) | FBG (mmol/L) | BMI (kg/m$^2$) | Dose (IU) |
|---|---|---|---|---|
| **All patients** | | | | |
| Baseline | 8.7 ± 1.4 | 11.2 ± 3.1 | 29.0 ± 4.7 | 13.7 ± 7.0 |
| 9 months | 7.0 ± 1.0 | 7.3 ± 1.9 | 28.5 ± 4.8 | 20.3 ± 9.6 |
| **Group 1: BMI<25 kg/m$^2$** | | | | |
| Baseline | 8.5 ± 1.4 | 11.2 ± 3.3 | 23.3 ± 1.5 | 12.9 ± 6.2 |
| 9 months | 6.9 ± 1.0 | 7.1 ± 2.0 | 24.2 ± 2.8 | 18.5 ± 8.3 |
| **Group 2: BMI<25–30 kg/m$^2$** | | | | |
| Baseline | 8.6 ± 1.3 | 11.1 ± 2.9 | 27.4 ± 1.4 | 13.4 ± 6.4 |
| 9 months | 7.0 ± 0.9 | 7.2 ± 1.8 | 27.2 ± 2.6 | 19.7 ± 8.9 |
| **Group 3: BMI<30–35 kg/m$^2$** | | | | |
| Baseline | 8.8 ± 1.4 | 11.5 ± 3.1 | 32.0 ± 1.4 | 14.4 ± 7.2 |
| 9 months | 7.1 ± 1.0 | 7.4 ± 1.9 | 30.8 ± 3.3 | 21.2 ± 9.5 |
| **Group 4: BMI >35 kg/m$^2$** | | | | |
| Baseline | 8.9 ± 1.6 | 11.6 ± 3.6 | 38.8 ± 3.8 | 15.3 ± 8.2 |
| 9 months | 7.2 ± 1.1 | 7.6 ± 2.2 | 36.7 ± 5.7 | 23.4 ± 11.9 |

**Conclusion:** These data suggest that, in daily clinical practice, patient BMI should be considered when determining insulin dosing. Here we have shown that insulin glargine in combination with OADs is effective in treating patients with Type 2 diabetes inadequately controlled on OADs alone. These results are consistent with those seen in clinical trials.
*This study was supported by Aventis Pharma.*

## 740

**Efficacy of insulin glargine in patients with Type 2 diabetes at different stages of disease**

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**Background and aims:** Earlier introduction of insulin to patients with Type 2 diabetes can help achieve the stringent treatment targets needed to prevent diabetic complications. This 28-week, randomized, open-label, European study investigated the efficacy of once-daily insulin glargine (LANTUS®) plus once-daily glimepiride (Amaryl®) in patients at different stages of Type 2 diabetes.
**Materials and methods:** 624 patients poorly controlled on oral antidiabetic agents (OADs) were treated with insulin glargine in the morning (AM) or bedtime (PM) plus glimepiride (AM) to a target fasting blood glucose (FBG) of ≤5.5 mmol/L (≤100 mg/dL). Glimepiride doses depended on previous OADs (2 mg [n=62]; 3 mg [n=105]; 4 mg [n=60]).
**Results:** At baseline, there was no difference between all glimepiride groups in terms of age (63.3 ± 10.3 years), body weight (80.1 ± 14.0 kg), BMI (28.5 ± 4.1 kg/m$^2$) or C-peptide (1.5 ± 0.9 nmol/L; p=non-significant). Diabetes duration and history of previous OAD treatment was significantly shorter in patients on lower glimepiride doses implying an earlier stage of the disease; glycaemic control ($HbA_{1c}$ and FBG) was also significantly better in these patients. $HbA_{1c}$ and FBG at endpoint were similar between groups (Table). However, patients on lower glimepiride doses required smaller dose increases of insulin during the study and less insulin at endpoint, gained less weight at endpoint (2 mg: 0.5 kg; 3 mg: 1.1 kg; 4 mg: 2.9 kg) and had significantly less symptomatic (2 mg: 30.7%; 3 mg: 32.4%; 4 mg: 50.0%) and nocturnal (2 mg: 8.1%; 3 mg: 5.7%; 4 mg: 21.7%) hypoglycaemia. In general, insulin doses depended on BMI level. Patients with a higher BMI needed higher insulin doses (BMI <25 kg/m$^2$: 24.4 ± 13.6 IU; BMI ≥25 kg/m$^2$ but <30 kg/m$^2$: 32.7 ± 16.1 IU; BMI ≥30 kg/m$^2$ but <35 kg/m$^2$: 39.9 ± 18.0 IU; BMI ≥35 kg/m$^2$: 39.5 ± 18.5 IU; p <0.0001).
**Conclusion:** The combination of insulin glargine and glimepiride results in good glycaemic control, regardless of the initial glimepiride dose or baseline $HbA_{1c}$. Patients on higher glimepiride doses (and thus at a later stage of the disease) and with higher BMI required higher insulin doses to achieve glycaemic targets.

| | | Glimepiride dose (mg) 2 | 3 | 4 | p value* |
|---|---|---|---|---|---|
| $HbA_{1c}$, % | Baseline | 8.5 ± 0.8 | 8.7 ± 0.9 | 9.2 ± 1.1 | p=0.0003 |
| | Endpoint | 7.1 ± 1.0 | 7.1 ± 1.1 | 7.3 ± 1.3 | p=0.6306 |
| Fasting blood glucose, mmol/L (mg/dL) | Baseline | 9.3 ± 1.9 (167 ± 35) | 9.9 ± 2.1 (179 ± 37) | 11.4 ± 2.7 (206 ± 49) | p<0.0001 |
| | Endpoint | 6.4 ± 1.3 (115 ± 27) | 6.5 ± 1.6 (117 ± 29) | 6.8 ± 1.8 (123 ± 32) | p=0.3064 |

*Between-treatment comparison
*This study was supported by Aventis Pharma.*

## 741

**Comparison of equivalent daily doses of biphasic insulin aspart and insulin glargine during isoglycaemic clamp studies in persons with Type 2 diabetes**

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**Background and aims:** The pharmacokinetic (PK) and pharmacodynamic (PD) properties of 0.5 U/kg biphasic insulin aspart (BIAsp30: 30% soluble and 70% protamine bound insulin aspart) and 0.5 U/kg insulin glargine (IGlarg) were compared during two 24 h isoglycaemic glucose clamp study days following bolus subcutaneous (sc) administration.
**Materials and methods:** The order of treatments was randomised. Patients with type 2 diabetes were administered a total daily dose 0.5U/kg of either insulin analogue. BIAsp30 (0.25 U/kg) was injected at 08.00 h (A) and 20.00 h (B) and IGlarg (0.5U/kg) was injected at 08.00 h, both by administration into the anterior abdominal wall. Plasma glucose was measured at 10-minute intervals throughout the 24 h clamp period and isoglycaemia was maintained by variable infusion of glucose (20%). Glucose infusion

rate (GIR), plasma insulin and C-peptide concentrations were estimated throughout each 24 h study period.
**Results:** All 12 patients with type 2 diabetes were male; mean (SD) age 58.3 (8.3) years, BMI 31.7 (3.4) kg/m$^2$, HbA1 c 7.3 (0.9) %. The plasma glucose remained constant during the clamp (CV: BIAsp30 6.3%; IGlarg 4.3%). Following injection of BIAsp30 GIR increased rapidly reaching peaks (incremental) of 2.2 (0.4) and 1.8 (0.5) mg.kg.min at 5 h 20 min and 3 h 20 min following first and second injection respectively, compared to IGlarg reaching a flatter peak of 1.8 (0.2) mg.kg.min at 11 h 30 min. GIR were essentially similar between 8–13 h and 19–24 h with the end of study (24 h) GIR at 0.8 (0.2) and 0.7 (0.1) mg.kg.min for BIAsp30 and IGlarg respectively. The overall GIR (AUC0–24 h) was 2.5 (0.4) for BIAsp30 and 1.9 (0.1) g.kg for IGlarg ($p<0.01$). The incremental plasma insulin peaks were 173 pmol/l reached at 2 h 45 min (A) and 198 pmol/l at 1 h 45 min (B) for BIAsp30, and 92 pmol/l at 12 h 15 after IGlarg, with a similar increment of 38 pmol/l at 24 h for both regimens. The overall plasma insulin (AUC0–24 h) following BIAsp30 was significantly greater (28%) than after a single injection of IGlarg (4.5 nmol/l.h and 3.5 nmol/l.h, respectively, $p<0.01$). Following injection of BIAsp30, plasma C-peptide fell from 0.91 ± 0.10 nmol/l to a nadir of 0.75 ± 0.09 nmol/l at 4 h 30 min (A), then recovered to 1.07 ± 0.13 nmol/l at 12 h before falling to 0.66 ± 0.08 nmol/l at 15 h 45 min (B) then rising to 0.72 ± 0.10 nmol/l at the end of the study. Plasma C-peptide following injection of IGlarg remained essentially unchanged (0.92 ± 0.10 nmol/l) throughout the study period.
**Conclusion:** During a 24 h isoglycaemic clamp both GIR and plasma insulin profiles were approximately 30% greater following subcutaneous injection of equivalent daily doses (0.5U/kg) of BIAsp30 given as 2 split doses compared to IGlarg given as a single dose. Suppression of endogenous insulin secretion was seen following the 2 injections of BIAsp30 but not following injection of IGlarg.

## 742

**Importance of basal insulin in uncontrolled Type 2 diabetic patients on intensive insulin regiments**

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**Background and aims:** Insulin glargine is biosynthetic insulin analogue with a prolonged duration of action compared with human NPH insulin. This study compared insulin glargine with NPH insulin in poorly controlled type 2 diabetic patients who had been previously treated with multiple daily injections of NPH insulin and regular insulin.
**Materials and methods:** This study was a randomized, cross-over, parallel-group study in which 62 type 2 diabetics were randomized to receive insulin glargine prebedtime plus lispro preprandially (LIS/GLAR) or NPH insulin prebedtime plus regular insulin preprandially (R/NPH) for up to 28 weeks. Dose titration of basal insulin was based on home measurements of blood glucose profiles 7 times a day. The fasting blood glucose (FBG) target was 6.7 mmol/L (120 mg/dl). A total of 54 subjects completed the study.
**Results:** Compared with R/NPH therapy, LIS/GLAR was associated with lower mean blood glucose levels (LIS/GLAR vs R/NPH): fasting (7.6 vs 9.2 mmol/l, $p<0.0001$), 2-h post breakfast (7.9 vs 10.6 mmol/l, $p<0.0004$), 2-h post lunch (8.1 vs 9.7 mmol/l, $p<0.001$) and 2-h post evening (7.8 vs 10.2 mmol/l, $p<0.001$). The mean changes in HbA1 c were −1.5% with LIS/GLAR (baseline 8.76) and −0.5% with R/NPH therapy (baseline 8.62)(treatment difference $p<0.05$; 95% CI −1.10, −0.7). Incidence of nocturnal hypoglycaemia on overnight profiles was 34% lower on LIS/GLAR compared with R/NPH therapy. Total insulin dose required to achieve target blood glucose control was lower on LIS/GLAR (1.31 IU/kg) compared with R/NPH therapy (1.66 IU/kg, $p<0.005$), although in most of the subjects in R/NPH therapy we couldn't achieve target blood glucose control.
**Conclusion:** Use of insulin glargine compared with NPH is associated with less hypoglycaemia and lower fasting and postprandial glucose levels when combined with analog insulin. Insulin glargine is more effective to maintain glycemic control in uncontrolled type 2 diabetic patients on multiple injection regimens.

## 743

**Fasting blood glucose for insulin adjustment with insulin glargine or NPH insulin combined with sulphonylurea in Type 2 diabetes**

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**Background and aims:** In the treatment of Type 2 diabetes, two basal insulins – insulin glargine (LANTUS®; GLAR) or NPH insulin (NPH) – are most commonly combined with oral antidiabetic agents (OADs). Insulin dosage is determined by fasting blood glucose (FBG). The aim of this abstract was to assess the reliability of FBG measured in the last 4 days compared with FBG achieved in the last month of a 6-month study.
**Materials and methods:** In a 6-month, multicentre, multinational study, metabolic control was compared using morning or bedtime insulin glargine or bedtime NPH, all combined with 3 mg glimepiride (Amaryl®). Efficacy and safety data for this study have been reported elsewhere. In a subgroup of German patients (GLAR: n=121, NPH: n=59; age: GLAR: 60 ± 9 years, NPH: 63 ± 9 years [p=0.04]; duration of diabetes: GLAR: 9 ± 6 years, NPH: 9 ± 5 years [p=0.86]; body mass index: GLAR: 29.9 ± 4.1 kg/m$^2$, NPH: 29.9 ± 4.2 kg/m$^2$ [p=0.97]), FBG in the last month of the study was compared with measurements in the last 4 days. In this period there was no dosage adjustment of insulin.
**Results:** The average FBG in the last month of the study was not different between the treatment groups (combined GLAR group: 7.4 ± 1.9 mmol/L [133 ± 34 mg/dL] vs NPH insulin group: 7.0 ± 1.7 mmol/L [126 ± 31 mg/dL]; p=0.2). With NPH insulin, the mean FBG during the last 4 days was different to the mean FBG during the last month of the study (6.7 ± 1.6 mmol/L [120 ± 29 mg/dL] vs 7.0 ± 1.7 mmol/L [126 ± 31 mg/dL]; p=0.002), whereas with insulin glargine there was no difference (morning GLAR: 7.3 ± 2.0 mmol/L [132 ± 36 mg/dL] vs 7.4 ± 1.9 mmol/L [133 ± 34 mg/dL]; bedtime GLAR: 7.4 ± 2.2 mmol/L [133 ± 39 mg/dL] vs 7.4 ± 1.9 mmol/L [133 ± 34 mg/dL]; p >0.2). There was a strong correlation of the average FBG of the last 4 days with the $HbA_{1c}$ at the end of the study (GLAR: $R^2$: 0.37, $p<0.001$; NPH: $R^2$: 0.35, $p<0.001$) and with the average FBG of the last month (GLAR: $R^2$: 0.88, $p<0.001$; NPH: $R^2$: 0.81, $p<0.001$).
**Conclusion:** After 6 months of therapy, the mean FBG over 4 days gives a similar estimate for the glycaemic control over the preceding month, which is more stable with insulin glargine than NPH insulin. Therefore, averaged FBG levels over 4 days can be used as a basis for insulin glargine dosage adjustments.
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## 744

**Starting insulin for Type 2 diabetes with insulin glargine added to oral agents versus twice-daily premixed insulin alone**

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**Background and aims:** When oral antidiabetic agents (OADs) no longer maintain good glycaemic control in patients with Type 2 diabetes, OADs are often stopped and twice-daily premixed insulins substituted. However, glycaemic targets ($HbA_{1c}$ ≤7.0%) are frequently not reached due, in part, to the risk of hypoglycaemia. The aim of this study was to compare, in these poorly controlled patients, the efficacy and safety of adding once-daily insulin glargine (LANTUS®) with continued OADs (insulin glargine plus OADs) versus switching to twice-daily premixed insulin.
**Materials and methods:** A 24-week, multicentre, randomized, open, parallel-group study in 364 insulin-naïve patients with fasting blood glucose (FBG) ≥6.7 mmol/L (≥120 mg/dL) and $HbA_{1c}$ 7.5–10.5% on OADs (glimepiride [Amaryl®] or other sulphonylurea plus metformin) compared once-daily morning insulin glargine plus OADs or premixed 30% regular/70% human NPH insulin twice daily (PreMix) monotherapy. Insulin dosage was titrated to target FBG ≤5.6 mmol/L (≤100 mg/dL) with insulin glargine plus OADs and to both FBG ≤5.6 mmol/L (≤100 mg/dL) and pre-dinner BG ≤5.6 mmol/L (≤100 mg/dL) with PreMix, using a weekly forced-titration algorithm.
**Results:** Mean age (60.6 ± 8.9 years), diabetes duration (9.9 ± 6.8 years), body mass index (29.5 ± 3.6 kg/m$^2$) and baseline $HbA_{1c}$ (8.84 ± 0.92%) were similar in both groups. Endpoint mean insulin dose was 28.2 IU daily with insulin glargine plus OADs versus 64.5 IU with PreMix. By intent-to-treat analysis, the $HbA_{1c}$ decrease from baseline was greater with insulin glargine plus OADs versus PreMix (−1.64 vs −1.30%; p=0.0003), and more

patients reached $HbA_{1c}$ ≤7.0 without documented nocturnal hypoglycaemia <3.3 mmol/L (<60 mg/dL) with insulin glargine plus OADs (45 vs 29%; p=0.0013). Similarly, FBG decreased more from baseline with insulin glargine plus OADs (–3.1 mmol/L [–56 mg/dL] vs –2.2 mmol/L [–39 mg / dL]; p <0.0001). Insulin glargine plus OAD-treated patients had fewer documented (<3.3 mmol/L [<60 mg/dL]) hypoglycaemic episodes (mean total event rate/patient: 1.9 vs 4.5; p <0.0001) with less weight gain (1.4 vs 2.1 kg; p=0.08) compared with patients treated with premixed insulin.
**Conclusion:** Adding once-daily insulin glargine to OADs achieves glycaemic control more effectively and with less risk of hypoglycaemia than the common practice of switching from OADs to twice-daily premixed insulin.
*This study was supported by Aventis Pharma Deutschland GmbH.*

## 745

### Insulin glargine allows dosing flexibility in patients treated to target $HbA_{1c}$ of ≤ 7%

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**Background and aims:** In patients with Type 2 diabetes, insulin glargine (LANTUS®) facilitates treating to target $HbA_{1c}$ of ≤7%. The aim of this study was to compare the effect of morning versus bedtime administration of insulin glargine in combination with glimepiride (Amaryl®) in patients with Type 2 diabetes.
**Materials and methods:** Type 2 diabetes patients (n=624) with poor glycaemic control ($HbA_{1c}$ 7.5–10.5%) using oral antidiabetic agents (OADs) were treated with once-daily insulin glargine (LANTUS®) in the morning (AM; n=312) or at bedtime (PM; n=312) plus glimepiride (2, 3 or 4 mg) in a 28-week, randomized, open-label, multinational European study. Insulin doses were adjusted using a forced titration algorithm to a target fasting blood glucose (FBG) of ≤5.6 mmol/L (≤100 mg/dL); target $HbA_{1c}$ was ≤7.0%.
**Results:** A total of 149 out of 312 (47.8%) patients in the AM group and 143 out of 312 (45.8%) patients in the PM group reached $HbA_{1c}$ ≤7.0% at endpoint. Baseline demographics of these patients were similar: mean age 61.6 ± 10.6 years versus 60.7 ± 8.8 years; diabetes duration 8.5 ± 5.2 years versus 9.4 ± 6.1 years; duration of OAD therapy 6.3 ± 5.0 years versus 6.5 ± 5.2 years (AM vs PM; all p=not-significant [NS]). Baseline mean weight was 80.7 ± 14.7 kg versus 83.3 ± 13.5 kg, body mass index (BMI) was 28.2 ± 3.9 kg/m$^2$ versus 28.8 ± 3.8 kg/m$^2$, $HbA_{1c}$ was 8.6 vs 8.5%; all p=NS. Results of patients who achieved $HbA_{1c}$ ≤7.0% are reported. These patients achieved a baseline to endpoint reduction in FBG that was not different between the two groups (AM: 10.9 to 6.3 mmol/L [196 to 114 mg/dL], Δ= 4.6 mmol/L [82 mg/dL]; PM: 10.6 to 6 mmol/L [191 to 108 mg/dL], Δ=4.6 mmol/L [83 mg/dL]; p=NS) and $HbA_{1c}$ (AM: 8.6 to 6.3%, Δ=2.3%; PM: 8.5 to 6.4%, Δ=2.1%; p=NS). Baseline to endpoint changes in insulin doses were comparable between the groups (16.4 to 33.0 IU vs 16.0 to 31.0 IU, AM vs PM; p=NS). The proportion of patients who had nocturnal hypoglycaemic episodes (AM: 12.8% vs PM: 16.8%; p=NS), confirmed symptomatic hypoglycaemia (AM: 49.7% vs PM: 42.0%) and severe hypoglycaemia (AM: 1.3% vs PM: 0.0%; p=NS) was also similar between the groups.
**Conclusion:** Insulin glargine, a basal long-acting human insulin analogue, can be administered with equivalent efficacy either in the morning or in the evening owing to its smooth time-action profile and near 24-hour duration of action. In this study, almost 50% of patients with Type 2 diabetes treated with insulin glargine plus glimepiride reached target $HbA_{1c}$ of ≤7% within 28 weeks, irrespective of morning or bedtime insulin glargine administration. This target was achieved with no difference in the number of patients experiencing nocturnal hypoglycaemic episodes between the two groups.
*This study was supported by Aventis Pharma.*

## 746

### Superfine Humulin Suspension (SHS, a novel human insulin formulation) and insulin glargine; exhibit a longer and flatter glucodynamic profile than NPH insulin

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**Background and aims:** Superfine Humulin Suspension (SHS) is a candidate for a once-a-day basal insulin that is developed to have a flat glucodynamic profile and prolonged duration of action. This 24-hr euglycemic glucose clamp-study compared the glucodynamic effect of 3 doses (each given once; 0.2, 0.4 and 0.8 U/kg) of SHS to a single dose (0.4 U/kg) of neutral protamine Hagedorn (NPH) insulin in healthy subjects. To gain preliminary understanding of how an established basal insulin would perform in our experimental system compared to NPH, a small group of subjects underwent the study with one dose (0.5 U/kg) of insulin glargine (Lantus®).
**Materials and methods:** This was an open label, randomized, 4-period, incomplete block, crossover study. We enrolled 25 healthy subjects (4 females and 21 males, age 22–43, BMI 18.6–27.3 kg/m$^2$) and randomized them to receive 4 different treatments by subcutaneous injection in 4 separate periods. There were at least 5 days between each study period.
**Results:** As expected, with increasing doses, SHS demonstrated a linear increase in total glucose consumption (Gtot) and peak effect (Rmax) from 0.2 to 0.8 U/kg. The duration of action, as indexed by late 50% TRmax (time to half maximal glucose infusion rate after Rmax), was similar for all 3 doses of SHS. At the same dose (0.4 U/kg) as NPH, SHS demonstrated a similar Gtot and Rmax. All 3 doses of SHS had a statistically significant prolonged duration of action when compared to NPH, the late 50% TRmax for SHS 0.2, 0.4 and 0.8 U/kg were approximately 15%, 19% and 25%, respectively, longer than NPH 0.4 U/kg. Glargine also demonstrated a prolonged duration of action and a delayed peak glucodynamic effect when compared with NPH.
**Conclusion:** The results show that SHS has a dose dependent response in total glucose consumption and peak effect and demonstrated consistently longer duration of action than NPH.

Least Squares Geometric means (90% Confidence Interval) of Glucodynamic Parameters

| Parameters | SHS (0.2 U/kg) | SHS (0.4 U/kg) | SHS (0.8 U/kg) | NPH (0.4U/kg) | Glargine (0.5 U/kg) |
|---|---|---|---|---|---|
| No. of Subjects | 20 | 21 | 21 | 23 | 8 |
| Rmax (mg/min) | 93.2 (75.4, 115) | 156 (127, 192) | 254 (206, 313) | 173 (141, 212) | 173 (126, 236) |
| Gtot (g) | 54.5 (44.1, 67.4) | 110 (89.4, 136) | 192 (156, 236) | 109 (89.4, 134) | 138 (100, 189) |
| Late 50% Trmax (min) | 1099 (988, 1222) | 1133 (1021, 1256) | 1190 (1072, 1320) | 952 (862, 1052) | 1349 (1144, 1591) |
| TRmax (min) | 574 (468, 703) | 490 (402, 597) | 601 (493, 733) | 452 (374, 546) | 768 (557, 1059) |

# PS 64

# Long-acting insulins in Type 2 diabetes

## 747

**Glycaemic control and hypoglycaemia in Asian Type 2 diabetes patients treated with insulin glargine plus glimepiride versus NPH insulin plus glimepiride**

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**Background and Aims:** Insulin glargine (LANTUS®), a basal, long-acting insulin analogue, has demonstrated improved efficacy and a reduced incidence of hypoglycaemia compared with NPH insulin in several populations. However, comparative data are not available for Asian patients. Thus, this study investigated the effect of bedtime insulin glargine compared with bedtime NPH insulin (NPH) on metabolic control and safety in Asian patients with Type 2 diabetes inadequately controlled on oral antidiabetic agents ($HbA_{1c}$ ≥7.5% and ≤ 10.5%).
**Methods and materials:** In this open-label, randomized, parallel group, multinational, multicentre, 24-week study, 443 Asian patients were treated with either once-daily insulin glargine (n=220) or NPH (n=223) at bedtime, plus glimepiride (Amaryl®). The predefined titration regimen started insulin at 0.15 IU/kg/day and titrated the dose upwards by 2 IU every 3 days until the target fasting blood glucose (FBG) of ≤6.67 mmol/L (≤120 mg/dL) was reached. Primary efficacy data aimed to demonstrate non-inferiority in the change from baseline in $HbA_{1c}$: one-sided 95% confidence interval (CI) (the lower limit of the two-sided 90% CI) for the difference between mean treatment effect size (insulin glargine-NPH insulin) should be ≥–0.4% (non-inferiority region); and the rate of hypoglycaemic episodes. This abstract focuses on the results of the main efficacy endpoint of the study.
**Results:** Baseline characteristics were similar between the insulin glargine and NPH groups (mean age: 55.6 ± 8.4 vs 55.6 ± 8.7 years; body mass index [BMI]: 24.8 ± 3.1 vs 25.1 ± 3.3 kg/m²; $HbA_{1c}$: 9.02 ± 0.88 vs 9.05 ± 0.84% [± standard deviation]). $HbA_{1c}$ levels decreased in the insulin glargine and NPH groups over the study period (–0.99 vs –0.77%). The difference between adjusted means was 0.19% (90% CI: 0.02, 0.36), demonstrating non-inferiority between the two treatments in the per-protocol population. A superiority analysis, in which the difference between the adjusted mean changes in the two treatment groups was 0.22% (95% CI: 0.02, 0.42) demonstrated the superiority of insulin glargine (p=0.0319) in the intent-to-treat population. Moreover, the number of all hypoglycaemic episodes was significantly lower in the insulin glargine versus NPH groups (number of episodes 682 vs 1019; p=0.0036), particularly for severe (5 vs 28; p=0.0257) and nocturnal (221 vs 620; p <0.0001) episodes. These results were achieved with a mean daily increase in insulin dose from 9.6 to 32.1 IU for the insulin glargine group versus 9.8 to 32.8 IU for the NPH insulin group.
**Conclusion:** In this study we have shown that insulin glargine provides better glycaemic control than NPH insulin with significantly fewer hypoglycaemic events, particularly severe and nocturnal, in Asian patients with Type 2 diabetes.
*This study was supported by Aventis Pharma.*

## 748

**Treatment with insulin glargine of patients with Type 2 diabetes in clinical practice: metabolic control over 30 months**

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**Background and aims:** The importance of insulin therapy in Type 2 diabetes is recognized to help achieve near normoglycaemia. However, barriers to insulin initiation and treatment success include fear of injections and weight gain. We performed an analysis of near-normal weight patients with Type 2 diabetes who had received insulin glargine (LANTUS®), a once-daily basal insulin analogue, for 30 months in clinical practice.
**Materials and methods:** Patients with Type 2 diabetes (n=46; 59% male; mean age 61.5 ± 8.6 years; mean body weight 86.3 ± 17.7 kg) pre-treated with oral antidiabetic agents (OADs; n=18) or insulin only (n=28) received insulin glargine, plus previous OAD or prandial insulin, and were observed for 30 months. The dose of insulin glargine was titrated according to the morning fasting blood glucose (FBG; 4.5–6.7 mmol/L [80–120 mg/dL]). Before initiation of insulin glargine, patients took part in an educational programme on insulin and diet at baseline, and had regular physician consultations throughout. Metabolic control was evaluated at 9, 18, 24 and 30 months. All variables were analysed using a paired *t*-test, from which mean values and standard deviation were derived.
**Results:** Overall, a significant reduction in $HbA_{1c}$ occurred from baseline to 30 months, and this decrease was observed after 9 months' treatment (Table). There was no change in weight from baseline to endpoint. When results were analysed by pre-treatment, both patients pre-treated with OADs or insulin only showed a significant reduction in $HbA_{1c}$ at 30 months (Table). Again, this change was observed after 9 months' treatment. No unexpected adverse events or episodes of severe hypoglycaemia were detected.
**Conclusion:** Treatment with once-daily insulin glargine, in combination with educational support, significantly improves metabolic control in Type 2 patients in clinical practice over 30 months.

| $HbA_{1c}$ (%) | All Type 2 | OAD pre-treated | Insulin only pre-treated |
|---|---|---|---|
| Baseline | 8.14 ± 1.7 | 9.16 ± 1.7 | 7.59 ± 1.5 |
| 9 months | 7.45 ± 0.9 | 7.28 ± 1.0 | 7.50 ± 1.0 |
| Change from baseline at 9 months | –0.69; p <0.002 | –1.88; p <0.002 | –0.09; p <0.001 |
| 30 months | 7.18 ± 0.9 | 6.90 ± 0.3 | 7.21 ± 1.1 |
| Change from baseline at 30 months | –0.96; p <0.001 | –2.26; p <0.001 | –0.38; p <0.001 |

## 749

**Simplifying treat to target – the LANMET study**

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**Background and aims:** We aimed to determine: 1) if good glycaemic control can be achieved using a simple insulin initiation protocol with few (every 3 month) visits, utilizing modem-assisted glucose monitoring and self-adjustment of the insulin dose; 2) if the daily insulin dose can be predicted and; 3) if insulin glargine (LANTUS®, GLAR) is associated with less hypoglycaemia than NPH insulin (NPH) when combined with metformin.
**Materials and methods:** In this multicentre, open, parallel-group study, 110 insulin-naïve patients with Type 2 diabetes (body mass index [BMI] 32 ± 1 kg/m², age 57 ± 1 year, $HbA_{1c}$ 9.5 ± 0.1%) were randomized and treated for 9 months with a fixed dose of 2 g metformin and either once-daily bedtime GLAR or NPH, titrated to achieve a target FPG of ≤5.5 mmo/L (≤100 mg/dL). Patients were instructed on how to monitor their fasting plasma glucose (FPG) and adjust their insulin dose by increasing it by 2 IU every 3 days, if all FPG values were above target. Glucose measurements were transferred to the treatment centre by modem. Patients were called every 2 weeks and seen every 3 months to encourage dose adjustment.
**Results:** At 9 months, FPG values (n=1269/last 3 months) averaged 5.72 ± 0.06 mmol/L and 6 ± 0.06 mmol/L (103 ± 1 and 108 ± 1 mg/dL) and insulin dose 68 ± 5 and 70 ± 6 IU in the GLAR and the NPH groups, respectively (not significant [NS], range 20–194 IU). Mean plasma glucose was significantly lower in the GLAR than the NPH group before dinner (8.6 ± 0.28 vs 10.1 ± 0.28 mmol/L [155 ± 5 vs 182 ± 5 mg/dL]; p=0.002) and after dinner (11.22 ± 0.28 vs 12.28 ± 0.28 mmol/L [202 ± 5 vs 221 ± 5 mg/dL]; p <0.03), respectively. $HbA_{1c}$ averaged 7.1 ± 0.1% in both groups. Symptomatic hypoglycaemia was 44% more frequent with NPH than with GLAR (8.0 vs 5.5 episodes/patient-year, p <0.05). Weight gain was less with GLAR than NPH (average 2.6 ± 0.6 and 3.5 ± 0.7 kg, respectively [NS]). Three baseline parameters independently predicted 49% (p <0.0001) of the variation in insulin dose at 9 months: weight, liver fat and baseline $HbA_{1c}$. (Alanine aminotransferase [ALT] positively correlated with the extent of liver fat measured by proton spectroscopy in 154 volunteers [0.55; p <0.0001] and baseline $HbA_{1c}$). The formula: Basal Insulin Dose (IU/d) =–175 + 1.21*weight (kg) + 0.366*ALT (U/L) + 12.7*$HbA_{1c}$ (%) predicts that an increase of 10 kg in weight, 33 U/L in ALT or 1.0% in $HbA_{1c}$ results in a 12 IU increase in insulin dose. This formula can be used to target basal insulin dosage requirements, and provides a method to adjust the insulin dose in patients with Type 2 diabetes depending on changes in these parameters.
**Conclusions:** Good glycaemic control can be achieved using a simple basal insulin plus metformin regimen with infrequent visits. Using modem-assisted glucose monitoring, patients can self-monitor and self-adjust basal

insulin dosing. The basal insulin dose needed to achieve the glycaemic target can be predicted as a linear function of weight, ALT and $HbA_{1c}$. Use of GLAR is associated with better pre- and post-dinner glycaemic control, and results in significantly less hypoglycaemia than NPH.
*This study was supported by Aventis Pharma.*

## 750

**Initiation of insulin glargine in patients with Type 2 diabetes sub-optimally controlled on once- or twice-daily NPH insulin: results from the AT.LANTUS trial**

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**Background and aims:** NPH insulin has traditionally been the intermediate-acting insulin of choice for patients with Type 2 diabetes. However, NPH insulin often requires twice-daily injection to provide adequate insulin cover and is associated with hypoglycaemia. The basal, long-acting insulin analogue insulin glargine (LANTUS®) is associated with better glycaemic control than NPH insulin at a similar rate of hypoglycaemia and weight gain. The AT.LANTUS study determined the best method for initiating and maintaining insulin glargine therapy by comparing two treatment algorithms in Type 2 patients. The aim of this subanalysis study was to assess glycaemic improvement of once-daily insulin glargine in patients previously on NPH insulin.
**Materials and methods:** This was a 24-week, multinational (n=59), multicentre (n=611), randomized, open study. Algorithm 1 was a visit-based titration using 2–8 IU increments (10 IU initiation dose for insulin-naïve patients). Algorithm 2 involved patient self-titration of 2 IU every 3 days (first dose based on FBG for insulin-naïve patients). Titration was based on target FBG of ≤5.5 mmol/L (≤100 mg/dL). Algorithms were compared for incidence of severe hypoglycaemia (patient requiring assistance and BG <2.8 mmol/L [<50 mg/dL]), other hypoglycaemic events and baseline to endpoint change in $HbA_{1c}$, FBG, body weight and insulin dose. This abstract reports results from patients switched from once- or twice-daily NPH insulin monotherapy to once-daily insulin glargine monotherapy.
**Results:** Overall, 4961 patients with Type 2 diabetes (52% female, median age 57.6 years [range 21–87 years], mean $HbA_{1c}$ 8.9 ± 1.3%, body mass index [BMI] 29.0 kg/m$^2$, diabetes duration 12.3 ± 7.2 years; duration of insulin pre-treatment 5.1 ± 5 years) were changed from their previous antidiabetes treatment. A subset of 362 patients (7.3%) were previously taking basal (87.3% NPH) insulin only (n=90 once daily; n=272 twice daily). After changing to once-daily insulin glargine (alone), there was a baseline to endpoint increase in daily insulin glargine dose of 12.1 IU, from 26.9 IU to 39.0 IU. FBG decreased from 8.6 mmol/L (155.1 mg/dL) to 5.7 mmol/L (102.7 mg/dL; −2.9 mmol/L [−52.4 mg/dL], p <0.001). There was no change in body weight (baseline: 71.0 kg; endpoint: 71.4 kg). When baseline to endpoint reductions in $HbA_{1c}$ were analysed by treatment algorithm for patients previously treated with NPH insulin, a significant decrease in $HbA_{1c}$ was observed with both algorithm 1 (from 8.8% to 8.0%) and algorithm 2 (from 9.0% to 8.1%); p <0.001. There was no significant difference in the incidence of severe hypoglycaemia between algorithms (1.4 vs 1.8%).
**Conclusion:** This study shows the low incidence of severe hypoglycaemia and improved $HbA_{1c}$ of once-daily insulin glargine in patients previously receiving once- or twice-daily NPH insulin, even with aggressive insulin titration regimens, and demonstrates that patients at an advanced stage of disease can safely and effectively self-manage titration of insulin glargine with an additional 0.9% reduction in $HbA_{1c}$.
*This study was supported by Aventis Pharma.*

## 751

**Initiation of basal insulin glargine therapy in Type 2 patients inadequately controlled on oral antidiabetic agents: results from the AT.LANTUS trial**

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**Background and aims:** For many patients with Type 2 diabetes, oral antidiabetic agents (OADs) do not provide adequate metabolic control, necessitating insulin therapy. However, fear of hypoglycaemia is a major barrier to initiating and appropriately titrating insulin therapy. This study investigated the optimal method to initiate and maintain insulin glargine (LANTUS®) therapy using two treatment algorithms (Algs) in Type 2 patients. This subanalysis established the effect on glycaemic control of initiating once-daily insulin glargine therapy in patients poorly controlled on OADs.
**Materials and methods:** This was a 24-week, multinational (59 countries), multicentre (611), randomized study. Alg1 was a visit-based titration using 2–8 IU increments (10 IU initiation dose for insulin-naïve). Alg2 involved patient self-titration of 2 IU every 3 days (first dose based on fasting blood glucose [FBG] for insulin-naïve). Titration was based on target FBG ≤5.5 mmol/L. Algs were compared for incidence of severe hypoglycaemia (requiring assistance and blood glucose <2.8 mmol/L), other hypoglycaemic events, baseline to endpoint change in $HbA_{1c}$, FBG, body weight and insulin dose. This abstract reports results for patients failing OAD combination therapy who were switched to once-daily insulin glargine plus ≥1 OADs.
**Results:** A total 962 (19.4%) patients of the treated 4961 were poorly controlled on OAD combination therapy: 340 received insulin glargine + 1 OAD; 525 received insulin glargine + >1 OAD. For patients receiving insulin glargine + 1 OAD, there was a significant decrease in $HbA_{1c}$ (−1.4%; p<0.001) from 9.1% to 7.9% with Alg1 and from 9.1% to 7.5% with Alg2 (−1.2% Alg1 vs −1.6% Alg2, p=0.03). FBG reduced by 4.0 mmol/L (from 10.2 mmol/L to 6.2 mmol/L; p<0.001), with superior reductions for Alg2 (−4.4 mmol/L) vs Alg1 (−3.6 mmol/L). Daily insulin glargine dose increased from 10 IU to 31.9 IU with Alg1 and from 12.2 IU to 46.1 IU with Alg2 (p<0.001). In the insulin glargine + >1 OAD group both arms showed a significant decrease in $HbA_{1c}$, with superior reductions with Alg2 (−1.82% from 9.2% vs −1.48% from 9.1% in Alg1 [p<0.005]). FBG reduced by 4.1 mmol/L (from 10.2 mmol/L to 6.1 mmol/L; p<0.001), with superior reductions for Alg2 (−4.4 mmol/L vs −3.7 mmol/L). Daily insulin glargine dose increased from 10.2 IU to 30.1 IU with Alg1 and from 11.6 IU to 34.5 IU with Alg2 (p<0.001). 42.7% of patients in Alg2 achieved $HbA_{1c}$ ≤7% compared to 31.1% in Alg1. Incidence of severe hypoglycaemia for both groups was <1%.
**Conclusion:** This study shows that in a large population with >12 years duration of diabetes, initiation and titration of once-daily insulin glargine safely facilitates early use of basal insulin therapy in primary (general practice) and secondary (hospital) care. The results show that insulin glargine allows patients at an advanced stage of diabetes to simply and effectively participate in the management of their treatment to achieve ≥1.8% reduction in $HbA_{1c}$, with the potential to significantly reduce the burden of care on healthcare professionals.
*This study was supported by Aventis Pharma.*

## 752

**The use of basal insulin infusion in a glucose clamp alters the late glucodynamic profile of NPH insulin**

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The euglycaemic glucose clamp aims to maintain euglycaemia through glucose infusion after the administration of insulin. This technique is widely used for the determination of insulin sensitivity and pharmacodynamic profiles of administered insulin. Variations exist in the conduct and analysis of such experiments; glucose infusion rate (GIR) adjustments may be automated or manual and subjects are clamped to a fixed or individualized baseline. A basal insulin infusion is often used, with the intent of suppressing endogenous insulin secretion. However this may influence the

glucodynamic assessment of the study insulin. The study objectives were to assess the effect of a concomitant continuous intravenous (IV) infusion of insulin lispro (LP) on the glucodynamics and pharmacokinetics of subcutaneous (SC) NPH insulin.
**Materials and methods:** An open label, randomised, four period crossover study, comparing:
A) Continuous IV infusion of normal saline for 24 hrs
B) NPH (0.4 U/kg) by SC injection given 2 hrs after the start of an IV infusion of normal saline
C) Continuous IV infusion of insulin LP (0.15 mU/kg/min)
D) NPH (0.4 U/kg) by SC injection, given 2 hrs after the start of an IV infusion of insulin LP (0.15 mU/kg/min).
Euglycemia was maintained for up to 24 hrs, with samples also assayed for serum immunoreactive insulin (for endogenous insulin and NPH), insulin LP and C-peptide concentrations. Insulin LP was used to distinguish the infused insulin from the endogenous and NPH components.
**Results and Discussion:** Mean 24 hr glucodynamic profile for Treatment B (NPH) had the TRmax and late $TR_{50\%}$ occurring at 6.8 hours and 16.3 hours respectively. After a slow rise to steady state, Treatment C (LP) showed a plateau of glucose demand up to 16 hours. For Treatment D (NPH+LP), the mean TRmax occurred at a similar time as for NPH alone (6.0 hours) but late mean $TR_{50\%}$ occurred later, at 19.3 hours from the time of NPH dosing. For appropriate comparison of techniques, individual profiles were adjusted by subtraction of appropriate control profiles (saline, LP). The mean control subtracted ((B-A) vs (D-C)) profiles for NPH up to 840 min post are similar. After 840 min, however, the GIR profiles appear to diverge. The $GIR_{24\,h}$ (77.3 mg/min vs 20.9 mg/min, p=0.001), and the $Gtot_{(840-1320\,min)}$ (37447 mg vs 15374 mg, p=0.011) were significantly higher for (D-C) than (B-A). The late $TR_{50\%}$ also appeared prolonged for (D-C), although this was not statistically significant (p=0.194).
**Conclusions:** When comparing glucodynamics data from studies employing different clamp methods, care must be taken to ensure that effects due to baseline insulin infusions are accounted for. The use of basal insulin infusion in this study did not appear to alter the glucodynamic profile of NPH for up to about 840 min post-dose, but increased glucose demand in later hours, which could potentially lead to overestimation of the duration of the effect.

Comparison of Baseline Corrected Glucodynamic Parameters

| Parameter | Treatment | LS Geometric Mean | (D-C) / (B-A) Ratio (90% C.I.) | p-value for difference |
|---|---|---|---|---|
| $R_{max}$ (mg/min) | (B-A) | 172 | 1.05 (0.87, 1.26) | 0.668 |
| | (D-C) | 180 | | |
| Late $TR_{50\%}$ (min) | (B-A) | 871 | 1.12 (0.97, 1.29) | 0.194 |
| | (D-C) | 975 | | |
| Gtot (mg) | (B-A) | 104533 | 1.16 (0.92, 1.45) | 0.277 |
| | (D-C) | 120753 | | |
| $Gtot_{(840-end)}$ (mg) | (B-A) | 15374 | 2.44 (1.43, 4.16) | 0.011 |
| | (D-C) | 37447 | | |

## 753

**Reproducibility of serum insulin and glucose infusion rate profiles of insulin glargine compared with NPH insulin and insulin ultralente**
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**Background and aims:** The intra-day fluctuation, poor reproducibility in serum insulin concentrations (INS) and glucose-lowering effect of insulins with peak action profiles, such as NPH insulin and ultralente, has stimulated the search for a 24-hour peakless basal insulin, of which insulin glargine (LANTUS®) is a paradigm.
**Methods and materials:** In this parallel, two replicate, single-dose, double-blind, randomized, euglycaemic clamp study, three groups of healthy male volunteers (n=12/group) aged 18–33 years received insulin glargine, NPH insulin or ultralente (0.4 IU/kg) by subcutaneous injection. A new analysis on individual intra-day variations in INS and glucose infusion rate (GIR) was performed. GIR was recorded every 10 minutes for 24 hours, and INS, corrected for endogenous INS, was determined hourly. Profile reproducibility was assessed using two methods: by between-day (absolute) differences in INS ($\Delta_{absolute}$-INS-cumulative [CUM]) and GIR ($\Delta_{absolute}$-GIR-CUM) and secondly, by individual standard deviation (SD) of diurnal between-day differences (raw) in INS (SD-$\Delta_{raw}$-INS) and GIR ($^{b}$SD-$\Delta_{raw}$-GIR).
**Results:** Although total insulin exposure ($INS\text{-}AUC_{0-24\,h}$) was ~25 to 35% greater for NPH insulin compared with ultralente and insulin glargine (ANOVA), the suppression of endogenous insulin release (C-peptide) and total glucose disposal ($GIR\text{-}AUC_{0-24\,h}$) were similar for all groups (Table).

| Point estimates between treatments (90% confidence interval)[a]/Ratio (median)[b] | Insulin glargine/NPH insulin | Insulin glargine/ultralente |
|---|---|---|
| $^{a}INS\text{-}AUC_{0-24\,h}$ | 0.64 (0.53, 0.77)# | 0.89 (0.74, 1.08) |
| $^{a}GIR\text{-}AUC_{0-24\,h}$ | 0.93 (0.67, 1.28) | 1.20 (0.83, 1.79) |
| $^{b}\Delta_{absolute}$-INS-CUM | 0.52#/[0.81c§] | 0.39# |
| $^{b}\Delta_{absolute}$-GIR-CUM | 0.94 | 0.60§ |
| $^{b}SD\text{-}\Delta_{raw}$-INS | 0.62#/[0.94c§] | 0.50§ |
| $^{b}SD\text{-}\Delta_{raw}$-GIR | 0.71§ | 0.49§ |

CUM=cumulative; Δ=difference; c: normalized for $INS\text{-}AUC_{0-24\,h}$; #p <0.05; (§p <0.05 excluding outliers)

Profile reproducibility assessed by between-day (absolute) differences in INS ($\Delta_{absolute}$-INS-CUM) and GIR ($\Delta_{absolute}$-GIR-CUM) were the same (when normalized) for insulin glargine and NPH insulin, but significantly larger for ultralente. When profile reproducibility was assessed by individual SD of diurnal between-day differences (raw) in INS (SD-$\Delta_{raw}$-INS) and GIR ($^{b}$SD-$\Delta_{raw}$-GIR), it was found that, on average, there was 30–50% less variation for insulin glargine GIR profiles as compared with NPH insulin and ultralente, although two subjects on insulin glargine were identified with ill-reproduced profiles in exposure, and, hence ill-replicated GIR.
**Conclusion:** Owing to its 24-hour peakless profile, the low day-to-day, within-subject variations in glucodynamic effect predict an improved reproducibility of basal glucose control with insulin glargine.
*This study was supported by Aventis Pharma.*

## 754

**Treatment with insulin detemir in combination with oral agents is associated with less risk of hypoglycaemia and less weight gain than NPH insulin at comparable levels of glycaemic improvement in people with Type 2 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 24 weeks of treatment with insulin detemir (IDet) and NPH insulin (NPH). Basal insulin was given as add-on therapy to insulin-naive people with Type 2 diabetes, treated with one or two oral antidiabetic drugs (OADs).
**Materials and methods:** A total of 475 men and women received treatment (IDet: 237, NPH: 238, sex: 53% males. Clinical characteristics (mean ± SD) age: 60.8 ± 9.2 yrs, duration of diabetes: 9.7 ± 6.4 yrs, BMI: 28.9 ± 3.6 kg/m$^2$, $HbA_{1c}$: 8.6 ± 0.8%). Subjects administered insulin detemir or NPH insulin morning and evening in combination with OADs. Pre-breakfast and pre-dinner plasma glucose targets were ≤ 6 mmol/L (108 mg/dL) throughout the trial. Subjects were individually titrated based on self-measured plasma glucose levels, using a tight titration schedule and simple algorithms with specified dose adjustments. OAD treatment was kept unchanged during the trial.
**Results:** $HbA_{1c}$ decreased by 1.8% points, 95% CI [−1.97; −1.71] in the insulin detemir group and by 1.9% points 95% CI [−2.01; −1.78] in the NPH insulin group and was comparable between treatments after 24 weeks, (IDet: 6.6%, NPH: 6.5%, mean difference (IDet-NPH: 0.1% point, ns). More than 70% of subjects in both groups achieved a $HbA_{1c}$ level ≤ 7%. However, a significantly higher proportion of subjects in the insulin detemir group reached a $HbA_{1c}$ level ≤ 7% in the absence of hypoglycaemia (IDet: 25.7%, NPH 15.5%, p<0.01) within the last 12 weeks of treatment. There was no statistically significant difference in fasting plasma glucose (IDet: 6.6, NPH: 6.3 mmol/L, ns). The risk of overall and nocturnal (23:00 to 06:00) hypoglycaemia was 47% and 55% lower with insulin detemir than with NPH insulin (p<0.001) and only 1 major hypoglycaemic episode occurred with insulin detemir compared to 8 episodes with NPH insulin. The overall safety profile was similar for the two treatments. Within-subject variation in pre-breakfast and pre-dinner plasma glucose was significantly lower with insulin detemir than with NPH insulin (SD: 1.32 vs. 1.44 mmol/L, p<0.001). Body weight gain after 24 weeks was 1.6 kg lower with insulin detemir than with NPH insulin (1.2 vs. 2.8 kg, p<0.001).

**Conclusion:** Treatment with insulin detemir compared to NPH insulin as add-on to OAD therapy, using a tight titration concept, resulted in less within-subject variation in pre-breakfast and pre-dinner plasma glucose, lower risk of hypoglycaemia and less weight gain at a similar clinically relevant level of improvement in glycaemic control. This indicates that insulin detemir has therapeutic advantages when treating people with Type 2 diabetes to target $HbA_{1c}$.

*This study was sponsored by Novo Nordisk*

# PS 65
# Insulin therapy in Type 2 diabetes

## 755

**Diabetes patients' stated preference for insulin therapies: trading health for convenience**

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**Background and aims:** Effective glycemic control in diabetes frequently depends on regular administration of insulin, but many patients may find insulin injections burdensome. The purpose of this study was to quantify diabetes patient preferences for short-term treatment outcomes and the number of daily insulin injections.
**Materials and methods:** A total of 292 US patients with type 2 diabetes completed a stated-preference questionnaire that included a series of 12 hypothetical treatment choices. Each treatment alternative specified and varied the number of daily insulin injections (1–3 injections), in addition to fasting plasma glucose (FPG), glycosylated hemoglobin ($HbA_{1c}$), number of hypoglycemic events per month, and monthly cost of treatment (range, $50–$200).
**Results:** The responses to the questionnaire indicated that type 2 patients placed significant value on reducing the number of daily insulin injections and improving glucose control. Reducing the number of injections from 3 to 2 per day was half as important as reducing the number of injections from 2 to 1 per day. Improving glucose control from the worst level (FPG >170 mg/dL; $HbA_{1c}$ > 8%) to the suboptimal level (FPG 70–170 mg/dL; $HbA_{1c}$ 7–8%) was 6 times as important as improving glucose control from the suboptimal level to the optimal level (FPG 90–120 mg/dL; $HbA_{1c}$ < 7%). Reducing the number of injections from 2 to 1 per day was twice as important as improving glucose control from a suboptimal to an optimal level.
**Conclusion:** Patients with type 2 diabetes are willing to sacrifice glucose control in order to avoid injections. Noninvasive insulin delivery methods that facilitate better glucose control therefore have the potential to be preferred by patients and improve long-term treatment outcomes.

## 756

**Efficacy, insulin dose and antibody formation in a four-year trial comparing twice daily biphasic insulin aspart 30 with twice daily biphasic human insulin**

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**Background and aims:** Biphasic insulin aspart (BIAsp 30) is a recently introduced formulation of the rapid-acting human insulin analogue, insulin aspart, composed of a soluble (30%) and a protamine-bound (70%) phase of insulin aspart. This presentation is focused on the the long-term aspects of antibody formation and its relation to insulin dose and overall efficacy (blood glucose (BG), major hypoglycaemic episodes (MHE) and $HbA_{1c}$). Twice daily treatment with BIAsp 30 was compared with another twice daily treatment regimen with biphasic human insulin 30 (BHI 30), a biphasic formulation of human insulin with 30% soluble and 70% protamine-bound human insulin.
**Materials and methods:** One hundred and fifteen people with diabetes was followed for a 4-year period. Ninety four people completed the trial. Formation of cross-reacting antibodies (Ins-ab), ie. antibodies cross-reacting with insulin and insulin aspart, efficacy and insulin dose data are reported here.
**Results:**$HbA_{1c}$ remained steady at a similar level in both treatment groups throughout the trial, 8.15(1.09) (SD))% for BIAsp 30 vs 8.26(1.06) for BHI 30 at last visit. Insulin dose increased during the trial in both treatment groups, from 0.57(0.27) to 0.72(0.34) U/kg for BIAsp 30 and from 0.55(0.18) to 0.61(0.16) U/kg for BHI 30. The frequency of MHEs was similar in the two groups: 30 MHEs in the first 2 years with BIAsp30 and 29 with BHI 30; the corresponding numbers for the last 2 years were 25 and 31 MHEs, respectively. In the BIAsp 30 treatment group Ins-ab increased from 8.8(12.0)% to 18.7(21.8)% in the first 3 months but decreased to 11.4(15.7)% at last visit. The corresponding numbers for Ins-Ab in the BHI

30 group were 8.9(14.3), 9.5(15.1), and 8.5(13.5)% at the same time points. The Ins-ab changes did not seem to be associated with changes in insulin dose or $HbA_{1c}$.
**Conclusion:** Similar efficacy and safety was found out to 4 years for BIAsp 30 and BHI 30. Cross-reacting insulin antibodies showed a transient rise at 3 months with BIAsp 30, but changes in Ins-ab did not seem to correlate with changes in blood glucose control or insulin dose.

## 757

**Effects of 8 hour overnight constant insulin infusion on fasting plasma glucose in obese Type 2 diabetic patients**
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**Background and aims:** A considerable number of type 2 diabetic patients will not reach target for good metabolic control on diet and oral antidiabetic drugs. Therefore it will often be necessary to introduce insulin as alternative or additional treatment. One way of introducing insulin treatment is to start night time insulin injections and maintain oral treatment during the daytime. The rationale behind this is that endogenous glucose production is increased during night time. Systematic dose-effect studies in these patients are few.
The primary objective of this study was to evaluate the effect of 8-hour overnight constant subcutaneous infusion with different doses of a short acting insulin analog (NovoRapid®) on fasting plasma glucose (FPG) concentration in obese patients with type 2 diabetes.
**Materials and methods:** The trial was performed as a mono-center, open, cross-over study. Ten obese patients (BMI 30.9 ± 3.7) with type 2 diabetes participated in the trial. The patients were not well controlled on their present oral medication (mean: HbA1 c 9.8, range:(8–12.9). The study consisted of each three consecutive study periods each of tree days' duration where constant subcutaneous insulin infusion by mean of an insulin infusion pump (MiniMed®) took place from 23:00 to 07:00 hours. In the first period (A) all patients received the same dose (2 iu/hour). During the two following periods the patients were given an individually dose depending on the fasting plasma glucose on day 4 in the first period (1–2.5 IU/hour). The periods were separated by a wash-out period of four weeks.
**Results:** In period A, we found a highly significant reduction in FPG from day 1 (mean: 11.62 range: 7.3–15.2) to day 2 (mean: 5.45 range: 3.8–8.7) p= 0.0022 and to day 4 (mean: 4.55 range: 3.6–6.1) p=0.0003. Furthermore the reduction was clinical relevant with a mean reduction of 7 mmol/l in fasting plasma glucose from day 1 to day 4. Moreover despite a great variation in plasma glucose just before the pump was started the final FPG were similar. Simultaneous with the rise in plasma aspart insulin concentration, a reduction in endogen insulin levels took place. The results in period B and C was combined with the results in period A by an ANOVA including day, dose and fasting FPG from day 4 in period A as covariates. The result demonstrated no period effect and no statistical difference between the effects obtained by the different doses on fasting plasma glucose.
**Conclusion:** 8-hour nightly subcutaneous infusion with a short acting insulin analog (NovoRapid®) is a safe and efficient way of introducing insulin treatment as a supplement to oral antidiabetic drugs in obese type 2 diabetic patients.

## 758

**Insulin treated patients with Type 2 diabetes mellitus have higher rates of nocturnal than daytime hypoglycaemia: continuous blood glucose monitoring (CBGM) run-in data from the REACH study**
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**Background and aims:** Few published data exist on CBGM in type 2 diabetes. Data generated from the run-in period of a randomised, double-blind crossover study were used to provide an overview of CBGM profiles.
**Materials and methods:** 84 male and 38 female adult patients with type 2 diabetes treated with insulin were enrolled into this study. During an 8-week run-in period insulin doses were adjusted to optimise blood glucose levels. CBGM was performed for 3 days at the end of the run-in period. 104 patients were using a BD premix regimen at baseline, 6 were using BD long-acting insulin and 12 were using other regimens. Mean BMI was 29.9 kg/m$^2$, mean age 63.1 years, and mean duration of diabetes 11.3 years.
**Results:** The overall rate of hypoglycaemic measurements (BG<3.5 mmol/l) in the period of monitoring was 4.63%. The mean rate of nocturnal hypoglycaemia (midnight to 0600) was 8.54%. The mean rate of daytime hypoglycaemia was 3.35%. A trend was observed towards increasing frequency of hypoglycaemia with lowering HbA1 c.
**Conclusion:** CBGM reveals higher rates of hypoglycaemia in type 2 diabetes than previously seen. Nocturnal hypoglycaemia detected using CBGM was more than twice as frequent as daytime hypoglycaemia in these type 2 diabetes patients using insulin.
*This study was funded by Novo Nordisk Ltd.*

## 759

**Frequency of severe hypoglycaemia: indicator of quality of care in patients with Type 2 diabetes mellitus with insulin therapy?**
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**Background and aims:** A low frequency of severe hypoglycaemic events (SHE) in patients with diabetes Type 1 is a marker of a high standard of education and care. After participation in a structured teaching and treatment programme (TTP) SHEs in diabetes type 1 were reduced to the half (0.35 to 0.16/pat/y). In a similar way the incidence of SHEs was chosen as a marker of care quality in the German Disease Management Programme for Diabetes mellitus Type 2. Two questions should be answered in this study: 1) is the incidence of severe hypoglycaemia a suitable marker of quality of care? 2) it is possible to reduce SHEs after participation in a structured in-patient programme?
**Materials and methods:** In the 78 hospitals which are members of the AKD evaluations of patient education were performed in 4856 Type 2 diabetic patients (age 61.3 ± 10.6y; range 22.8–95.1; duration since diagnosis 10.6 ± 8.3y; range 0.6–65.0) from 1998 to 2004. HbA1 c (DDCT adjusted), body weight, blood pressure were recorded before an 12–15 month after participation in an in-patient TTP. In 2441 patients data on incidence of severe hypoglycaemia (injection of glucose or glucagon) were available.
**Results:** Fifty nine patients (2.7%) had 80 (range 1–4) SHEs before and 50 patients (1.9%) 84 (range 1–10) SHEs 12 month after TTP. The incidence was 0.0328 before and remained stable 12 months after TTP 0.034 (p=0.86). Out of the 59 patients with SHE before TTP 45 (2.1%) had no further SHEs, but 14 patients (0.57%) had 23 SHEs 12 months after TTP. However 35 (1.4%) patients without SHEs before TTP had 60 new SHEs 12 months after TTP. Patients with SHEs before TTP had a longer duration since diagnosis (13.9 ± 8.3 vs. 10.4 ± 8.2y; p=0.001), a lower body weight (80.2 ± 16.1 vs. 86.9 ± 17.0 kg; p=0.001) compared to patients without SHEs before TTP. But there was no difference in HbA1 c (8.11 ± 1.5 vs. 8.44 ± 1.84%; p=0.17), insulin dose (45.1 ± 27.6 vs. 44.4 ± 33.5 IU/d; p=0.91), frequency of blood glucose self-monitoring (15.3 ± 13.4 vs. 12.9 ± 12.3 tests per week; p=0.66). Patients with SHEs 12 month after TTP had a slightly lower HbA1 c (6.89 ± 1.35 vs. 7.31 ± 1.39%; p=0.036), lower age (57.41 ± 11.47 vs. 60.75 ± 9.93y; p=0.019), and lower frequency of self monitoring (4.2 ± 0.84 vs.18.95 ± 11.4 test per week; p=0.004), but no difference in duration since diagnosis (11.6 ± 9.7 vs. 10.5 ± 8.2y; p=0.34), insulin dose (51.7 ± 18.9 vs. 53.4 ± 45.0 IU/d; p=0.84) and body weight (82.3 ± 16.1 vs. 88.2 ± 17.1 kg; p=0.085) compared to patients without severe hypoglycaemia.
**Conclusion:** In contrary to patients with diabetes type 1 severe hypoglycaemic events are very rare in patients with type 2 diabetes. The incidence could not be reduced after participation in a structured teaching and treatment programme. The incidence of severe hypoglycaemia is no useful parameter for the measurement of the quality of care in Type 2 diabetic patients if the initial incidence is as low as in the population studied.

## 760

**Validation of a new questionnaire, better assessing the impact of hypoglycemia and level of glucose control on the QOL of insulin-treated diabetic patients**
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**Background and aims:** Hypoglycemia is an event in the daily life of insulin-treated diabetic patients that has a major impact on their QOL. Existing questionnaires routinely used to assess the QOL of diabetic patients do not demonstrate full impact of this dimension. A new questionnaire was recently developed and validated to better capture the dimensions of the QOL of diabetic patients: Burden of hypoglycemia and feeling of glycemic

control. The aim of this study was to evaluate the correlation between the different parameters of glycemic control and the hypoglycemia events and the score achieved with new QOL questionnaire.
**Materials and methods:** 153 insulin-treated diabetic patients were enrolled (42 patients with type 1 diabetes and 111 with type 2 diabetes, 71 males and 82 females; mean age: 58.7 years; average BMI: 23.3; average HbA1 c: 7.51%; diabetes treatment duration: 15.5 years). Questionnaires on QOL, perception of diabetes management and Diabetes Treatment Satisfaction Questionnaire (DTSQ) were randomly distributed to the patients under informed consent. Blood glucose levels measured by self-monitoring, and clinical information gathered by physicians during the last month were collected.
**Results:**
1. The newly developed QOL questionnaire was comprised of 32 items and was divided into four sub-domains by factor analysis: daily and social life, burden of hypoglycemia, feeling on glycemic control and impact of nocturnal hypoglycemia. The internal consistency coefficient (Cronbach $\alpha$) was 0.94.
2. QOL scores of type 1 diabetic patients were significantly lower than those of type 2 patients (t-test, p=0.000). QOL scores lowered as the number of injection increased (One-factor ANOVA, p=0.0045).
3. HbA1 c (Pearson analysis, r =–0.30, p=0.0002) and fasting blood glucose (FBG) level (r = –0.28, p=0.0006) showed a negative correlation with QOL score. Standard deviation of FBG level (r =–0.36) and of bedtime blood glucose level (r = –0.53) also showed negative correlation with QOL score.
4. Regarding the relation with hypoglycemia, the followings were observed:
1) The frequency of total hypoglycemia (r = –0.20), nocturnal hypoglycemia (r = –0.34), severe hypoglycemia (r = –0.24), nocturnal severe hypoglycemia (r = –0.24) and hypoglycemia unawareness (r = –0.20) showed a negative correlation with QOL scores ($p < 0.01$).
2) Regarding the time of day when hypoglycemia occurs, only hypoglycemia during sleep significantly lowered QOL (p=0.001).
3) When patients felt that their hypoglycemia was more severe, QOL was lower (p=0.00001). When patients had stronger confidence that they would not suffer from nocturnal hypoglycemia, their QOL score was higher (p=0.00001).
5. DTSQ didn't demonstrate any influence of these hypoglycemia events and feeling of glycemic control on treatment satisfaction.
**Conclusion:** This new questionnaire was shown to be useful for detecting the effects of glycemic control and hypoglycemia on diabetic patients' QOL. A good glycemic control was closely related with a good QOL. Larger fluctuations in blood glucose levels with hypoglycemia lowered QOL score. All kinds of hypoglycemia including nocturnal, severe and hypoglycemia unawareness increased the feeling of burden and lowered QOL. This new questionnaire will allow to assess the influence on QOL of new insulins, such as insulin glargine, which can achieve better glycemic control with less hypoglycemia.

## 761

**Risking health to avoid injections: stated preferences of Canadians with diabetes**
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**Background and aims:** Patients with diabetes may require regular administration of insulin to achieve adequate glycemic control, but many patients find insulin injections burdensome. The purpose of this study was to quantify diabetes patient preferences for short-term treatment outcomes and the number of daily insulin injections.
**Materials and methods:** A total of 1054 Canadian subjects with diabetes (type 1, n = 118; type 2, n = 936) completed a stated-preference questionnaire that included a series of 12 hypothetical treatment choices. Each treatment alternative specified and varied the number of daily insulin injections (1–3 injections using an insulin pen), fasting plasma glucose (FPG), glycosylated hemoglobin ($HbA_{1c}$), number of hypoglycemic events per month, and monthly cost of treatment (range, $50-$200 Canadian).
**Results:** Patients placed significant value on reducing the number of daily insulin injections and improving glucose control. On average, patients valued avoiding an increase from 1 injection to 2 injections. Likewise, patients valued improving their current glucose control to the best level (FPG, 4–7 mmol/L; $HbA_{1c} < 7\%$). Type 1 patients placed less value on a decrease in the number of injections than did type 2 patients. In contrast, type 1 patients placed greater value on improving glucose control than did type 2 patients. Insulin-using type 2 patients placed no value on reducing the number of injections while insulin-naïve type 2 patients placed significant value on reducing the number of injections. In addition, insulin-using type 2 patients placed greater value on improving glucose control than did insulin-naïve type 2 patients. While almost all patients were willing to sacrifice glucose control to avoid injections, type 2 patients were more than twice as likely as type 1 patients to do so. Among type 2 patients, insulin-naïve patients were more willing to sacrifice glucose control to avoid injections than were insulin-using patients.
**Conclusion:** The study results indicate that diabetes patients value reducing the number of injections from an insulin pen. Given the significant long-term health risks associated with poor glucose control, noninvasive insulin delivery methods that facilitate better short-term glucose control have the potential to improve long-term health outcomes, especially among type 2 patients.

## 762

**SIT vs CIT: a prospective, randomised cross-over-study to evaluate preference of therapy and treatment satisfaction in conventionally (CIT) and supplementarily preprandially (SIT) insulin therapy treated patients with diabetes mellitus Type 2**
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**Background and aims:** Multiple injection therapy (MIT/SIT) with preprandial normal insulin and, if necessary, intermediate or long acting insulin overnight, displaced conventional insulin therapy (CIT) with premixed normal and long acting insulin twice a day though superior results for glycemic control, weight gain or improved quality of life has not been convincingly shown by a randomised controlled study. To evaluate potential patients predilection and treatment satisfaction under either therapy the following study was conceived.
**Material and methods:** In a randomised prospective study 40 younger patients with diabetes mellitus type 2 after secondary therapy failure of treatment with oral antidiabetic drugs (OAD) and/or diet were randomised to receive CIT or MIT/SIT first and conducted each therapy for 8 weeks. Then after cross-over and additional teaching an other 8 weeks period ensued with the opposite therapy. At study end patients decided on their own before contacting their medical doctor which type of therapy they desired to continue. Total study duration 20 weeks, 4 weeks run-in, 8 weeks therapy 1, after cross-over 8 weeks therapy 2.
**Results:** Whole group: age 55,9 years, diabetes duration 7,7 years, weight, 84,5 kg, BMI 29,35 kg/m$^2$, Hba1 c 8,9% (normal mean 5,2%), 26 working, 14 retired or jobless. Groups after initial randomisation: CIT n=20/MIT/SIT n=20, age 57,3/54,7 years (p=0,25), diabetes duration 7,15/8,20 years (p=0,38), weight 86,1/82,9 kg (p=0,47), BMI 29,35 / 29,34 kg/m$^2$ (p=0,99), Hba1 c 8,67/9,23% (p=0,28), systolic blood pressure 137,6/137,5 mmHg (p=0,99), diastolic blood pressure 81,6/80,9 mmHg (p=0,82). During the study Hba1 c improved significantly (8,9 to 7,24%, p<0,0001), blood pressure did not change (syst. 137 to 136 mmHg, p=0,56; diast. 81 to 78 mmHg, p=0,34). Weight (85,5 to 86,3 kg, P=0,02) and BMI (29,35 to 29,98, p=0,02) increased significantly. 20 patients opted for CIT (working 13/ retired 7), 20 patinets for MIT/SIT (working 13/retired 7). 10 minor (CIT 4/ MIT/SIT 6) and no major (i.v. glucose or glucagon injection) hypoglycemic episodes occurred. 34 patients continued with their last type of therapy.
**Conclusion:** Both therapy variants improve glycemic metabolism significantly and are safe. Severe Hypoglycemia did not occur, minor in both types of therapy roughly equally. Patients did not prefer either therapy variant. Working or retirement did not influence patients therapy option. The majority maintained the last applied therapy variant.

## 763

**The demographic characteristics and the extent of the problems regarding the early onset of chronic complications at the start of insulin treatment in patients with Type 2 diabetes in Turkey**
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**Background and aims:** The goal of a multicentric study carried out in all Diabetes Centers in Turkey is to investigate the extent of the problem with the use of insulin in type 2 diabetics and its reflection to the patient and the association with the complications.

**Materials and methods:** The study population consisting of patients with type 2 diabetes using oral antidiabetic agents and whom the initiation of insulin therapy was decided was recruited from 105 Diabetes Centers in Turkey. According to this criterion 20 patients from each Center was selected randomly. 1529 patients (F/M 727/801, mean chr. age 57.5 ± 11.3yrs) were evaluated regarding their demographic characteristics and the macro- and microvascular complications according to the ADA criteria.
**Results:** The mean time intervals for starting the oral antidiabetic (OAD) vs insulin treatment were 0.69 ± 2.2yrs vs 9.94 ± 6.6yrs, respectively. The reason for starting the insulin treatment was the unresponsiveness to oral antidiabetic agents in 75.7% of patients, infection or other disease in 14.0%. At its initiation, 38% of the patients resisted the insulin treatment. The obesity rate was 75.2% (BMI >25 kg/m$^2$) (mean BMI 29.5 ± 5 kg/m$^2$ in female, 26.8 ± 4 kg/m$^2$ in male patients). At the start of insulin treatment 72.0% of patients had macroangiopathy, 61.3% had microangiopathy and 61.4% had biochemical abnormalities. Regarding the macroangiopathic complications, the prevalence of hypertension was 63.7%, coronary heart disease (CHD) 25.0%, myocard infarct (MI) 6.6% and diabetic foot 31.1%. The duration of DM was 7.2yrs in male and 7.0yrs in female patients at the diagnosis of hypertension; 9.1yrs and 10.4yrs at the diagnosis of CHD; 8.5yrs and 14.1yrs at the diagnosis of MI; 9.4yrs and 12.5yrs at the diagnosis diabetic foot. As to the microangiopathic complications, 37.4% of patients had diabetic retinopathy, 22.5% nephropathy and 43% neuropathy. The duration of DM was 11.5yrs in male and 12.1yrs in female patients at the diagnosis of diabetic retinopathy; 11.5yrs and 11.6yrs at the diagnosis of nephropathy; 9.4yrs and 10.2yrs at the diagnosis of neuropathy. Hypercholesterolemia was found in 50% and hypertriglyceridemia in 37.6% of patients.At the start of the insulin treatment, the mean duration of DM was 10.3yrs in patients with macroangiopathy and 7.5 yrs without any macroangiopathy; 11.1 yrs in patients with microangiopathy and 7.1yrs without any microangiopathy.
**Conclusion:** The delay in change from the OAD to insulin treatment is caused by the fear of both the patients and the doctors. A noticeable correlation was found between the macro- and microvascular complications and the late initiation of insulin treatment.

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# New approaches to lowering glucose

## 764

**Opposite effects of sodium tungstate on insulin and somatostatin secretion**

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**Background and Aims:** Oral tungstate administration normalizes hepatic glucose metabolism in streptozotocin diabetic rats. Tungstate displays insulin-like effects in normal hepatocytes. In rat pancreas, tungstate stimulates the insulin response to arginine and inhibits the somatostatin secretion induced by this amino acid. We have investigated: 1) the effect of tungstate on insulin and somatostatin responses to various secretagogues; and 2) the insulinotropic effect of tungstate in somatostatin-depleted pancreases.
**Materials and Methods:** The study was performed in the perfused rat pancreas. Cysteamine (300 mg/kg b.w.) was administered intragastrically 24 h before perfusions. Sodium tungstate was tested at 5 mmol/l. Hormones were measured by RIA.
**Results:** In normal pancreases, tungstate inhibited the somatostatin responses to 16.6 mmol/l glucose (incremental area: 80 ± 109, Mean±SEM, vs. 808 ± 246 pg/10 min in controls; $p<0.01$), to 30 nmol/l glucagon (335 ± 331 vs. 1559 ± 208 pg/10 min in controls; $p<0.025$) and to 10 mmol/l arginine (75 ± 18 vs. 1520 ± 320 pg/15 min in controls; $p<0.01$) and potentiated the insulin responses to these secretagogues (by approx. 40%). Cysteamine treatment induced a marked pancreatic somatostatin depletion (79 ± 8 vs. 295 ± 37 ng/g wet tissue) and a lack of somatostatin response to arginine (incremental area: 75 ± 181 pg/20 min vs. 1525 ± 355 pg/20 min in untreated rats; $p<0.01$). In these pancreases, the stimulatory effect of tungstate on arginine-induced insulin release was comparable to that observed in normal rats. Finally, in cysteamine-treated rats, tungstate failed to significantly modify basal somatostatin output ($F_{15,60}=2.03$; NS) and exerted an insulinotropic effect comparable to that found in normal pancreases (22 ± 5 vs. 21 ± 4 ng/15 min in controls).
**Conclusions:** 1) The potentiating effect of tungstate on insulin secretion reinforces the antidiabetic effect of this compound. 2) Tungstate infusion provides a new model of somatostatin secretion blockade. 3) Our results suggest that the insulinotropic effect of tungstate is not paracrine-mediated through its concomitant inhibition of somatostatin secretion.
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## 765

**Mechanism of hypoglycemic action of a herbal syrup CI002 in Type 2 diabetic Long-Evans rats**

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**Background and aims:** In an acute study we have previously reported that a plant material coded as CI002 showed significant hypoglycemic activity in type 2 diabetic model rats. With a view to explore the mechanism of action of the material the present study is undertaken to see its effect on glycemic, insulinemic and lipidemic status of type 2 diabetic model rats after chronic administration.
**Materials and methods:** Type 2 diabetes was induced by a single intraperitoneal injection of streptozotocin (90 mg/kg body wt) to 48 hours old pups. After 3 months of streptozotocin injection male rats, weighing between 180–200g, were used in this study. The rats were divided into 3 groups with (8 number in each group): i) Vehicle (receiving only water), ii) Treated with syrup of the plant material (0.3 ml/kg body wt) and iii) Treated with standard drug glibenclamide (5 mg/kg body wt). The rats were treated with the syrup twice daily for 28 consecutive days. 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. The parameters measured were serum glucose (by glucose-oxidase), serum fructosamine (enzymatic-colorimetic), serum insulin (by ELISA), lipid profile (by enzymatic- colorimetric) and liver glycogen (by Anthrone-reagent) method.
**Results:** Oral administration of the syrup for 28 days resulted in significant reduction in serum glucose level ($p<0.01$ on the 14th days and $p<0.001$ on

the 28th days) whereas the level of serum insulin significantly increased in type 2 diabetic model rats ($p<0.001$). Serum fructosamine level was found to be decreased by 40%. Among the atherogenic lipids LDL-cholesterol was found to be decreased significantly in the syrup group ($p<0.05$). Liver glycogen content was found to be increased by the syrup. The increase, however, was not significant. The response to glibenclamide was comparable to that of the syrup. Glibenclamide treated group ameliorated the diabetic condition to the same extent as syrup but did not show any effect on lipid levels.
**Conclusion:** The results suggest that CI002 may contain hypoglycemic principles which stimulate insulin secretion resulting in increased disposal of glucose.

## 766

**Abstract withdrawn**

## 767

**Abstract withdrawn**

## 768

**Antiatherogenic effect of new antioxidant L-2264 in diabetic rabbits**
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**Background and aims:** Increased risk of cardiovascular disease in diabetic patients may be partially related to abnormal lipid metabolism and oxidative damage. We have previously shown that a new derivative of triazolopirimidines L-2264 decrease lipid peroxidation and increase insulin sensitivity in rats with insulin resistance. The aim of the study was to evaluate the impact of long-term treatment with L-2264 on the glucose homeostasis and lipid profile 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 L-2264 (100 mg/kg/day per os) for 1 month. At the end of the experiment rats were subjected to a glucose tolerance test (GTT, 0.5 g/kg i.v.). Blood was sampled in a fasting state for analysis of glucose, plasma insulin, HbA1 c, fructosamine, serum total cholesterol (TC), triglycerides (TG), LDL-C, HDL-C and NEFA. Oxidative status of experimental animals was assessed by determination of hydroperoxides (diene, oxidiene and tetraene conjugates), and total antioxidant activity (TAA) in plasma.
**Results:** Administration of L-2264 decreased basal hyperglycaemia ($11.2 \pm 0.5$ vs D: $18.8 \pm 0.5$; C: $3.6 \pm 0.2$ mmol/l, $p<0.01$), improve glucose intolerance (area under curve over GTT was $685 \pm 29$ vs D: $1330 \pm 70$; C: $285 \pm 40$ mmol/l/min, $p<0.001$) compared to diabetic controls. L-2264 also decreased fructosamine ($1.7 \pm 0.2$ vs D: $3.0 \pm 0.2$; C: $0.9 \pm 0.1$ mmol/l, $p<0.01$) and HbA1 c levels 2-fold ($p<0.01$) in comparison with diabetic rabbits. The treatment with L-2264 provided reduction in NEFA ($0.7 \pm 0.02$ vs D: $1.6 \pm 0.05$; C: $0.4 \pm 0.01$ mmol/l, $p<0.001$), TG ($0.7 \pm 0.16$ vs D: $4.9 \pm 0.6$; C: $0.7 \pm 0.08$ mmol/l, $p<0.001$) and TC ($1.4 \pm 0.07$ vs D: $1.8 \pm 0.09$; C: $1.1 \pm 0.03$ mmol/l, $p<0.01$) levels compared to with diabetic controls. Furthermore L-2264-supplementation elevated HDL-C 1.6-fold ($p<0.01$) and significantly reduced LDL-C ($p<0.05$) compared to diabetic rabbits. The treatment with L-2264 reduced hydroperoxides contents 1.5–2-fold and increasing TAA 2-fold compared to non-treated diabetic group ($p<0.05$).
**Conclusion:** We suggest that L-2264 possesses antiatherogenic effect due to improvement of glycaemic control and lipid profile, attenuating lipid peroxidation and improving free radicals defense system in diabetic rabbits. These results may have implications in prevention of diabetic macrovascular complications.

## 769

**Novel antioxidant phensuccinal ameliorates metabolic disturbances in rates with fructose-induced insulin resistance**
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**Background and aims:** Accumulating evidence suggest a link between increased oxidative stress and insulin resistance. We have previously shown that the low-toxic succinate derivative Phensuccinal (Ph), which is currently in clinical trials as antioxidant, improves lipid profile in diabetic rabbits. The aim of the study was to explore the effect of Ph on insulin resistance and oxidative stress in fructose-fed rats.
**Materials and methods:** Male Wistar rats were divided into three groups: the control group (C, n=8), the high fructose-fed group (F, n=8), which had free access to 250 g/L solutions of fructose for 8 weeks and fructose-fed group treated with phensuccinal (F+Ph) for 8 weeks (25 mg/kg/day per os). At the end of the study fasted rats were subjected to the glucose tolerance test (GTT, 3 g/kg i.p.). Indexes of insulin resistance (IR) were determined using the homeostasis model assessment methods (HOMA) and quantitative insulin sensitivity check index (QUICKI). Serum levels of non-esterified fatty acids (NEFA), triglycerides (TG) and alanine aminotransferase (ALT) were measured as parameters of insulin resistance. Activity of hepatic glucose-6-phosphatase was also measured in all experimental groups. Oxidative status was estimated by lipid peroxidation intermediates - plasma thiobarbituric acid reactive substances (TBARS) and total antioxidant activity (TAA).
**Results:** Fructose feeding induced insulin resistance, as indicated by higher HOMA-IR (F: $4.64 \pm 0.70$ vs C: $1.50 \pm 0.01$, $p<0.001$) and lower QUICKI ($p<0.01$) indexes. The fructose group also developed glucose intolerance, hypertriglyceridemia, significantly elevated NEFA levels, ALT and glucose-6-phosphatase activities ($p<0.01$). Administration of Ph protected the forming of fructose-induced glucose intolerance. Area under curve over the GTT was F+Ph: $596 \pm 74$ mM × hour vs F: $992 \pm 61$, $p<0.01$; C:$561 \pm 49$. The treatment with Ph decreases insulin resistance (HOMA-IR index F+Ph: $2.53 \pm 0.18$, $p<0.01$), triglyceride concentration (F+Ph: $0.663 \pm 0.028$ vs F: $0.896 \pm 0.043$ mmol/l, $p<0.01$), NEFA levels ($0.99 \pm 0.08$ vs F: $4.14 \pm 0.25$ mmol/l, $p<0.001$) and serum ALT activity ($p<0.02$) in compared with fructose-fed rats treated with placebo. Activity of glucose-6-phosphatase was reduced by 45 % ($p<0.01$) in liver of fructose-fed animals after administration of Ph. In addition, the use of Ph was associated with decrease in TBARS concentration 2-fold ($p<0.001$) and increase in TAA by 25 % ($p<0.01$) compared to control fructose-fed rats.
**Conclusion:** We suggest that Ph administration prevents glucose intolerance development and reduce insulin resistance decreasing hypertriglycerdemia, NEFA levels, gluconeogenesis and oxidative stress in high fructose-fed rats. These results substantiate the potential of Phensuccinal as a new therapeutic agent for the correction of insulin resistance states.

# PS 67

# GLP-1 analogues

## 770

**Pharmacokinetics of an oral drug (paracetamol) administered at various times in relation to subcutaneous injection of exenatide (exendin-4) in healthy subjects**

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**Background and aims:** Exenatide is an incretin mimetic with potential glucoregulatory activity in patients with type 2 diabetes. Mechanisms of action include enhancement of glucose-dependent insulin secretion, glucose-dependent suppression of plasma glucagon levels, and slowing of gastric emptying.
**Materials and methods:** This randomized, single-blind, placebo (PBO)-controlled 6-way crossover study assessed the effect of exenatide on the absorption pharmacokinetics (PK) of an orally administered drug, paracetamol (PAR), when given either prior to, at the time of, or after exenatide injection. PAR was selected as a prototype for oral drugs that are absorbed in the small intestine, but not in the stomach. Forty healthy volunteers were randomized (age 41 ± 10 y; BMI 26.7 ± 3.0 kg/m$^2$; 53% female; 88% Hispanic) and 39 completed all 6 regimens on 6 different days. Oral PAR elixir (1000 mg) was ingested at 0 h on PBO day (PBO injected subcutaneously at 0 h) and at −1, 0, +1, +2, and +4 h on exenatide days (10 µg injected subcutaneously at 0 h). A standardized breakfast was ingested at +15 min each day. Mean plasma PAR concentrations were ≥0.25 µg/mL (lowest level of assay quantitation) within 15 min of ingestion in all treatment arms.
**Results:** Mean plasma PAR $AUC_{0-12\,h}$ values were similar, differing by no more than 25%, in all treatment arms (range: 40 to 53 µg · h/mL). Peak plasma PAR concentrations ($C_{max}$) were similar when PAR was ingested 1 h prior to exenatide injection (16.0 µg/mL) and when it was administered concurrent with PBO injection (16.7 µg/mL). Administration at all other test times resulted in a 37% to 56% reduction of PAR $C_{max}$ values. Time to reach $C_{max}$ ($T_{max}$) was delayed 0.3, 3.6, 2.7, and 1.0 h relative to PBO in the 0, +1, +2, and +4 h exenatide treatments arms, respectively, but was unchanged when PAR was ingested 1 h prior to exenatide injection. All regimens were generally well tolerated, with most frequent treatment-emergent adverse events being mild nausea and vomiting. One episode of moderate nausea led to withdrawal.
**Conclusion:** Exenatide concurrent with or preceding PAR ingestion slowed the rate of PAR absorption. The extent of PAR absorption was not affected by exenatide administration in a clinically significant manner.

## 771

**Effect of injection site on relative bioavailability of exenatide (exendin-4)**

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**Background and aims:** Exenatide is a 39-amino acid peptide belonging to a new class of antidiabetic agents, incretin mimetics, having glucoregulatory activity in patients with type 2 diabetes mellitus. Here we report the results of a randomized, open-label, crossover study designed to assess the relative bioavailability of subcutaneous exenatide injected into the arm or thigh compared with the abdominal injection site used in previous clinical trials.
**Materials and methods:** We evaluated 25 subjects with type 2 diabetes: age 56.2 ± 8.1 y; BMI 33.0 ± 5.1 kg/m$^2$; $HbA_{1c}$ 8.0 ± 1.7% (±SD). Subjects received a single 10 µg injection of exenatide followed by 10 h of plasma sampling.
**Results:** Geometric LS mean exenatide $AUC_{0\text{-}inf}$ values were 63935 ± 6608 pg · min/mL (abdomen;±SEM), 59573 ± 6157 pg · min/mL (arm), 62148 ± 6424 pg · min/mL (thigh). The AUC geometric LS mean ratio for arm vs abdomen was 0.93, with geometric 90% CI ratios of 0.82 to 1.05; and for thigh vs abdomen was 0.97, with geometric 90% CI ratios of 0.86 to 1.1. Consistent with the observed data, intrasubject variability was low among the three 10 µg SC treatments (coefficient of variation, 26%). Geometric LS mean exenatide $C_{max}$ values were 220 ± 24 pg/mL (abdomen), 218 ± 23 pg/mL (arm), and 193 ± 21 pg/mL (thigh). The $C_{max}$ geometric LS mean ratio for arm vs abdomen was 0.99, with geometric 90% CI ratios of 0.85 to 1.15, and for thigh vs abdomen was 0.88, with geometric 90% CI ratios of 0.75 to 1.02. The most common treatment-emergent adverse events were mild-to-moderate nausea, vomiting, and headache.
**Conclusion:** In summary, all injection sites yielded equivalent pharmacokinetic profiles strongly suggesting equivalent exenatide bioavailability after subcutaneous injection into the arm, thigh, or abdomen.

## 772

**Effects of exenatide on first and second phase insulin secretion in response to intravenous glucose in subjects with Type 2 diabetes**

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**Background and aims:** Exenatide (synthetic exendin-4) increases insulin secretion (IS) during hyperglycemia. The present study examined whether intravenous (IV) exenatide enhances 1st and 2nd phase IS in response to an IV glucose bolus in subjects with type 2 diabetes (T2DM).
**Materials and methods:** Fourteen subjects with T2DM [mean±SD: 2 F/11 M, age 56 ± 7 y, BMI 31.7 ± 2.5 kg/m$^2$, $HbA_{1c}$ 6.6 ± 0.7%; treated with diet/exercise alone (n=1), metformin (n=11), or acarbose (n=2)] were randomized to receive placebo (PBO) and exenatide sequentially (13 completed), and compared to 12 healthy subjects with normal glucose tolerance (3 F/9 M, age 57 ± 9 y, BMI 32.0 ± 3.0 kg/m$^2$) who did not receive exenatide. In subjects with T2DM, insulin was infused to reduce glucose to 80–100 mg/dL within ~3 h, at which time either exenatide or PBO infusion was initiated (300 min infusion). One-hundred eighty min after starting exenatide or PBO, and 30 min after discontinuing insulin, an IV glucose bolus (0.3 g/kg) was given. Glucose, insulin, C-peptide, and glucagon were measured, and IS was estimated by deconvolution analysis of C-peptide. Prior to the IV glucose bolus, glucose concentrations were ~95 mg/dL in all groups.
**Results:** Treatment of patients with T2DM with exenatide increased insulin ($P<0.005$) and C-peptide AUCs ($P<0.005$) during the 1st (0–10 min) and 2nd (10–120 min) phases of IS by ~2- to 4-fold, and also increased IS rates throughout the 120 min, relative to PBO. Exenatide-treated patients with T2DM had a similar secretory pattern, and higher IS rates than healthy subjects, in contrast to patients with T2DM treated with PBO, who had lower 1st phase IS rates than healthy control subjects. Glucagon concentrations fell after IV glucose in all groups, with no further lowering by exenatide.
**Conclusion:** These data demonstrate that IV exenatide enhances 1st and 2nd phase IS after IV glucose in patients with T2DM, mimicking the early secretory pattern of healthy subjects. This effect is consistent with an ability of exenatide to improve β-cell function acutely.

## 773

**Effects of exenatide (exendin-4) on glycemic control and weight in patients with Type 2 diabetes treated with metformin and a sulfonylurea**

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**Background and aims:** This study evaluated the ability of exenatide, an incretin mimetic, to improve glycemic control in patients with type 2 diabetes and hyperglycemia on maximal doses of metformin and a sulfonylurea (SFU).
**Materials and methods:** The design was a triple-blind, placebo (PBO)-controlled study with a 4-wk PBO lead-in and 30-wk treatment period. Subjects were randomized to 5 µg subcutaneous exenatide twice daily (BID; arms A and B) or PBO for 4 wks. Subsequently, arm A remained at 5 µg, arm B escalated to 10 µg. All subjects continued metformin, but to explore the risk of hypoglycemia, subjects were randomized to either maximally-effective (MaxED) or minimum-recommended (MinRD) SFU dose. The intent-to-treat population included 733 subjects (55 ± 10y, BMI 33.6 ± 5.7 kg/m$^2$, $HbA_{1c}$ 8.5 ± 1.0%; ± SD); 82% (10 µg), 84% (5 µg), and 76% (PBO) completed.
**Results:** Wk 30 $HbA_{1c}$ changes from baseline (±SE) were −0.77 ± 0.08% (10 µg), −0.55 ± 0.07% (5 µg), and +0.23 ± 0.07% (PBO; adjusted $P<0.001$ vs PBO). Mean PBO-adjusted $HbA_{1c}$ reductions were −1.0% (10 µg) and ~0.8% (5 µg). $HbA_{1c}$ ≤7% was achieved by 30% (10 µg), 24% (5 µg), and 7% (PBO) of subjects ($P<0.001$). For MaxED arm, $HbA_{1c}$ changed by −0.91 ± 0.11% (10 µg), −0.67 ± 0.10% (5 µg), and +0.16 ± 0.10% (PBO; adjusted $P<0.001$). For MinRD arm, $HbA_{1c}$ changed by −0.62 ± 0.10% (10

µg), −0.43 ± 0.10% (5 µg), and 0.30 ± 0.10% (PBO; adjusted $P \leq 0.001$). At Wk 30, both exenatide arms had significant weight loss from baseline (−1.6 ± 0.2 kg each exenatide arm, −0.9 ± 0.2 kg PBO; $P \leq 0.01$ vs PBO). Mild or moderate nausea was the most frequent adverse event (49% 10 µg, 39% 5 µg, 21% PBO). There was one episode of severe hypoglycemia (5 µg). The incidence of mild/moderate hypoglycemia was 28% (10 µg), 19% (5 µg), and 13% (PBO); and appeared lower with MinRD than with MaxED.
**Conclusion:** Exenatide was generally well tolerated and significantly lowered $HbA_{1c}$ with no weight gain in patients with type 2 diabetes unable to achieve adequate control with combined metformin-sulfonylurea therapy.

## 774

**Effect of exenatide (exendin-4) on glycemic control and safety over 30 weeks in sulfonylurea-treated patients with Type 2 diabetes**
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**Background and aims:** Exenatide (exendin-4) is an incretin mimetic with potential antidiabetic activity. The aim of this study was to evaluate the effects of exenatide on glycemic control and safety among patients with type 2 diabetes taking at least the maximally effective dose of a sulfonylurea (SFU).
**Materials and methods:** This was a triple-blind, placebo (PBO)-controlled, multicenter, 30-wk study, with a 4-wk, single-blind, placebo lead-in period, after which subjects were randomized to 5 µg subcutaneous exenatide twice daily (BID; arms A and B) or PBO for 4 wks. Subsequently, doses in arm A remained at 5 µg while doses in arm B were escalated to 10 µg BID. Subjects continued SFU therapy. Of the 377 subjects in the intent-to-treat population (ITT) (60% M, 55 ± 11 y, BMI 33.4 ± 5.6 kg/m$^2$, $HbA_{1c}$ 8.6 ± 1.2%), 260 (69%) completed the study (60% of PBO and 73% of exenatide subjects).
**Results:** At Wk 30, exenatide treatment resulted in significant reductions in $HbA_{1c}$ from baseline (10 µg: −0.9 ± 0.1%, 5 µg: −0.5 ± 0.1% vs. PBO: +0.1 ± 0.1%, $P<0.001$ for each exenatide arm, ITT). In the evaluable population, 41%, 33%, and 9% of subjects attained an $HbA_{1c} \leq 7\%$ (10 µg, 5 µg, and PBO, respectively). The 10 µg arm had a significant reduction in body weight from baseline to Wk 30 (10 µg: −1.6 ± 0.3 kg vs. PBO: −0.6 ± 0.3 kg, $P< 0.05$, ITT). Treatment-emergent adverse events were generally mild-to-moderate, with nausea and hypoglycemia being the most frequent. No severe hypoglycemia occurred.
**Conclusion:** Over 30 wks, exenatide significantly reduced $HbA_{1c}$ in patients with type 2 diabetes failing maximally effective doses of SFU. Exenatide was generally well tolerated and resulted in significant reductions in body weight at the highest dose.

## 775

**Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with Type 2 diabetes**
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**Background and aims:** This study evaluated the effects of exenatide, an incretin mimetic with potential antidiabetic activity, on glycemic control in patients with type 2 diabetes inadequately controlled with maximally effective doses of metformin.
**Materials and methods:** The design was a randomized, triple-blind, placebo (PBO)-controlled, 30-wk study. After 4 wks of PBO, subjects were randomized and began 4 wks of 5 µg subcutaneous exenatide twice daily (BID; arms A and B) or PBO. Subsequently, doses in arm B were escalated to 10 µg BID. All subjects continued metformin. There were 336 subjects in the intent-to-treat (ITT) population (age 53 ± 10 y, BMI 34.2 ± 5.9 kg/m$^2$, $HbA_{1c}$ 8.2 ± 1.1%) and 272 (81%) completed the study.
**Results:** At Wk 30, $HbA_{1c}$ changes from baseline were −0.78 ± 0.10% (10 µg), −0.40 ± 0.11% (5 µg), and 0.08 ± 0.10% (PBO; adjusted $P< 0.01$). Of evaluable subjects, 46% (10 µg), 32% (5 µg), and 13% (PBO) with baseline $HbA_{1c} > 7\%$ (n=243) achieved $HbA_{1c} \leq 7\%$ ($P<0.01$). Fasting and postprandial plasma glucose levels decreased in exenatide arms compared with PBO ($P<0.05$). Subjects in the exenatide arms had dose-dependent and progressive weight loss, with significant end of study reductions from baseline in both exenatide arms (10 µg: −2.8 ± 0.5 and 5 µg: −1.6 ± 0.4 kg vs PBO: −0.3 ± 0.3 kg, [$P<0.05$]). The most frequent adverse events were generally mild or moderate and gastrointestinal in nature. No severe hypoglycemia was observed. The incidence of mild or moderate hypoglycemia was 5.3%, 4.5%, and 5.3% in the 10 µg, 5 µg, and PBO arms, respectively.
**Conclusion:** Over 30 wks, exenatide significantly reduced $HbA_{1c}$ with no increase in the incidence of hypoglycemia in patients with type 2 diabetes failing maximally effective doses of metformin. Exenatide was generally well tolerated and resulted in reduced body weight.

## 776

**Exenatide: postprandial glucose pharmacodynamics at various dosing times relative to a meal in patients with Type 2 diabetes**
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**Background and aims:** Exenatide is an incretin mimetic under clinical investigation for improving glycaemic control in patients with type 2 diabetes using oral agents. This study assessed the postprandial (pp) pharmacodynamics (glucose and insulin), safety and tolerability of various dosing times of exenatide in relation to a standard breakfast in patients with type 2 diabetes.
**Materials and methods:** Eighteen subjects (16 M, 2F; 58 ± 6 yrs; BMI 29.2 ± 3.64 kg/m$^2$; HbA1 c 6.8 ± 0.61%) participated in this single centre, open label, placebo controlled, randomised, six-way crossover study. All antihyperglycaemic medications (except metformin) were discontinued at least 7 days before first dose. Subjects received subcutaneous (sc) injections of either placebo (−15 min) or 10µg exenatide at −60, −15, 0, +30, and +60 min times relative to a standardised meal over 6 consecutive days. Serial blood samples were taken for plasma glucose (PG) and plasma insulin (PI) measurements from −60 min to 360 min postmeal and were assessed relative to placebo.
**Results:** For all exenatide treatments, baseline-adjusted (BA) incremental pp glucose $AUC_{0-6\,h}$ was significantly reduced compared to placebo; BA pp minimum glucose concentrations (incremental $C_{min}$) were significantly lower compared to placebo and BA pp maximum glucose concentrations (incremental $C_{max}$) were significantly lower for all exenatide treatments except the +60 min administration that could not be distinguished from placebo. For exenatide treatments, the peak pp glucose concentration was lower when exenatide was administered premeal or at mealtime compared to postmeal administration. The duration of the pp glucose excursion above baseline for each exenatide treatment was less than that following placebo. Mean PI profiles in the −60, −15, and 0 min exenatide dosing groups were lower than placebo; mean PI increased relative to placebo for the postmeal dosing groups, which may be due to higher pp glucose concentrations for these treatments. Trends in PI secretion across treatments appeared consistent with exenatide's action of glucose-dependent insulin release. Transient low blood glucose concentrations that resolved without intervention were observed in 8 of 18 subjects when exenatide was administered postmeal. The most common treatment-emergent adverse events were mild to moderate nausea, vomiting and headache.

| Dosing Times (relative to meal) | Incremental $AUC_{0-6\,h}$ (mg·min/dL)** | Incremental $C_{min}$ (mg/dL** | Incremental $C_{max}$ (mg/dL)** |
|---|---|---|---|
| **Placebo (at −15min)** | 5556.14 | −41.61 | 103.76 |
| **− 60min** | −7607.15* | −57.52* | 25.11* |
| **− 15min** | −7182.36* | −62.60* | 27.53* |
| **0min** | −7468.32* | −68.15* | 33.60* |
| **+ 30min** | −4912.29* | −72.59* | 81.69* |
| **+ 60min** | −5336.71* | −77.72* | 92.62 |

*Statistically significant difference from placebo at alpha=0.05 (2-sided)
**Data are least square means

**Conclusions:** In this study, all exenatide treatments demonstrated an improvement in plasma glucose excursions compared to placebo. Premeal and with meal administration of exenatide exhibited greater reduction of pp glucose excursions compared to postmeal administration. These data support flexible dosing of exenatide within 60 min before or with a meal.

## 777

**Liraglutide, a long-acting GLP-1 analogue, reduces body weight and food intake in obese candy fed rats while a DPP-IV inhibitor, LAF237, does not**

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**Background and aims:** The combined effect of GLP-1 compounds on glucose and body weight homeostasis is promising for the treatment of type 2 diabetes. However, native GLP-1 has a very short half-life. Liraglutide is a long-acting GLP-1 analogue with pharmacokinetic properties suitable for once daily injection. Significant reductions in blood glucose, food intake and body weight has been shown in many animal models. DPP-IV inhibitors inhibit the degradation of a variety of bioactive peptides, some of which are in the glucagon family, i.e. GLP-1. DPP-IV inhibitors have clearly been shown to reduce glucose whereas it is more unclear what happens to food intake and body weight. We have studied the difference on food intake and body weight of a long-acting GLP-1 analogue (liraglutide, $t^1/_2$=12 h in man) and a long-acting DPP-IV inhibitor (LAF237) using obese rats fed a diet of chow and 5 daily alternating kinds of candy, in order to mimic the excessive caloric intake in obese humans.
**Materials and methods:** We used 12 weeks treatment with liraglutide (0.2 mg/kg s.c. bid, n=10) and LAF237 (10 mg/kg p.o. bid, n=10) in obese rats fed a diet of chow and 5 daily alternating kinds of candy. The rats had free choice between chow and candy. Food intake and body weight were measured daily.
**Results:** The liraglutide treated rats had a significant reduction in body weight, compared to vehicle treated obese rats (−14.2 ± 4.2 g vs. +25.0 ± 2.5 g, p=0.0001). Liraglutide treatment normalized the bodyweight of the obese rats to the level of a lean control group (301 ± 9.7 g vs. 309 ± 4.9 g, p=ns). LAF237 treated rats had a weight gain comparable to vehicle (+24.3 ± 6.0 g vs. +26.0 ± 2.5 g, p=ns) and the body weight at the end of the study was significantly higher than the lean control group (341 ± 2.1 g vs 309 ± 4.9 g, p=0.01). Liraglutide significantly (p=0.009) reduced total cumulated caloric intake. This reduction was a selective reduction in calories obtained from candy (p=0.001), since there was actually an increase in calories obtained from chow (p=0.017). No difference was found between LAF237 and vehicle treatment in caloric intake of obese rats.
**Conclusion:** In conclusion, liraglutide normalized the bodyweight of obese candy fed rats and selectively reduced candy-derived caloric intake, whereas LAF237 showed no effect on bodyweight or caloric intake in obese candy fed rats.

## 778

**Liraglutide as add-on to metformin in type 2 diabetes: significant improvement in glycaemic control with a reduction in body weight compared with glimepiride**

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**Background and aims:** Liraglutide (NN2211) is a GLP-1 derivative designed for once-daily administration (s.c.). This trial investigated the effect on glycaemic control and body weight of liraglutide as monotherapy or as add-on to metformin (met) compared with met monotherapy or met+glimepiride (glim).
**Materials and methods:** One hundred and forty-four patients with type 2 diabetes were enrolled; 63% male, mean (±SD) age 56 (8.6) years, fasting serum glucose (FSG) 13.3 (2.6) mM, $HbA_{1c}$ 9.4 (1.0) % and body weight 93 (13.2) kg. After 2–6 weeks forced met titration to 1 g BID, patients with an FSG ≥ 9 mM were equally randomized to a 5-week double-blind treatment period. The dose of liraglutide was increased weekly from 0.5 mg OD to a maximum dose of 2 mg OD in steps of 0.5 mg.
**Results:** Repeated measurement analyses for FSG and body weight are shown in the Table.

| Comparison (difference) | FSG (mM) | 95% CI | Weight (kg) | 95% CI |
|---|---|---|---|---|
| liraglutide+met vs. met | −3.90 | [−5.0;−2.9] | −0.4 | [−1.2;0.3] |
| liraglutide+met vs. met+glim | −1.25 | [−2.3;−0.3] | −2.9 | [−3.6;−2.1] |
| liraglutide vs. met | −1.37 | [−2.4;−0.3] | −0.4 | [−1.1;0.4] |

With baseline values being similar, the liraglutide + met group was the only group to have a mean decrease in $HbA_{1c}$ larger than 1% points (−1.1, 95% CI: −1.3; −0.8%) after 5 weeks. There were no biochemically confirmed episodes of hypoglycaemia with liraglutide treatment (mono or add-on to met). Nausea was the most prominent GI side effect but led to withdrawal in only 4% (3/72) of patients exposed to liraglutide.
**Conclusions:** Liraglutide can be used at doses up to 2 mg/day when weekly up-titration is used. As add-on to met, (1) liraglutide leads to superior glycaemic control compared with glim; (2) despite better glycaemic control, weight is lost (liraglutide −2.4%) rather than gained (glim +0.9%); (3) GI side effects, although reported frequently, were rated acceptable and rarely interfered with continuation of liraglutide treatment.

# PS 68

# Non-insulin analogues

## 779

**Neutral endopeptidase 24.11 inhibition improves glucose tolerance in anaesthetised pigs independently of effects on active glucagon-like peptide-1**

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**Background and aims:** We have previously shown that the neutral endopeptidase (NEP) 24.11 inhibitor, candoxatril, further improves the anti-hyperglycaemic and insulinotropic effects of glucagon-like peptide-1 (GLP-1) in anaesthetised pigs when given in combination with the prototypal dipeptidyl peptidase IV (DPP IV) inhibitor, valine-pyrrolidide. However, the role of NEP 24.11 in incretin hormone metabolism *in vivo* is still unclear, and it is unknown whether inhibition of NEP 24.11 alone may also potentiate the effects of GLP-1. This study examined whether candoxatril had any effect on exogenous GLP-1 metabolism or its insulinotropic or anti-hyperglycaemic effects.
**Materials and methods:** Non-fasted, anaesthetised pigs received two successive i.v. GLP-1 infusions (0.75 pmol/kg/min), one alone and one in the presence of candoxatril (5 mg/kg, given 60 min after the end of the first GLP-1 infusion). An i.v. glucose load (0.2 g/kg) was given during each GLP-1 infusion.
**Results:** Administration of candoxatril significantly reduced NEP 24.11 activity, and increased C-terminal GLP-1 steady-state arterial concentrations (from 48 ± 12 to 143 ± 19 pmol/l; P = 0.002) and plasma half-life (from 2.6 ± 0.3 to 9.4 ± 0.8 min; P < 0.0001). However, plateau concentrations (27 ± 1 to 30 ± 3 pmol/l) and the half-life (1.4 ± 0.1 to 1.6 ± 0.1 min) of intact, biologically active, GLP-1 were unaffected by candoxatril. The glucose excursion was significantly reduced ($\Delta AUC_{0-67\ min}$, 100 ± 5 vs 69 ± 6 min × mM, P = 0.023) and the glucose elimination rate was increased (from 6.6 ± 0.5 to 8.5 ± 0.5 %/min, P = 0.0478) in the presence of candoxatril. Immunoreactive insulin levels were not changed by candoxatril ($\Delta AUC_{0-67\ min}$, 3067 ± 477 vs 3240 ± 658 min × pM, P = 0.690).
**Conclusion:** Candoxatril improves the metabolic stability of C-terminal GLP-1 immunoreactivity *in vivo*, although the peptide is still degraded by endogenous DPP IV, resulting in unchanged pharmacodynamics for intact, biologically active GLP-1. We conclude that candoxatril improves glucose tolerance, by mechanisms which are independent of changes in active GLP-1 concentrations, and suggest that NEP 24.11 inhibition may be useful in diabetes treatment.
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## 780

**CJC-1131, a long acting GLP-1 analog for Type 2 diabetes mellitus: clinical development update**

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**Background and aims:** CJC-1131 is a synthetic GLP-1 analog being developed for the treatment of type 2 diabetes (T2DM) based on Drug Affinity Complex (DAC) technology. After a single injection, covalent binding to albumin results in a long, 10-day, half-life in human subjects. A preliminary 14 to 20 day, ascending daily dose, placebo-controlled study in 25 T2DM patients treated with a 2, 4, 8 or 12 µg/kg demonstrated a dose and duration-dependent improvement in glycemia and body weight. We now report interim results from a 12-week open label monotherapy study in T2DM patients designed to evaluate the efficacy and safety of different doses and administration regimens.
**Materials and methods:** The study included approximately 150 evaluable observations. Following drug washout, patients entered an initial 4-week CJC-1131 ascending dose titration phase, followed by random allocation to one of four treatment regimens, for an 8-week treatment with either daily, 2 or 3 CJC-1131 injections per week, or once weekly administration. Additional patients were allocated to a no-treatment control group. The primary endpoint is AUC glucose in standardized meal tests (SMT) at the end of each period, and HbA1 c was also measured every other week. Plasma biochemistries, plasma levels of CJC-1131 and IgG-IgE specific antibodies were analyzed at central laboratories.
**Results:** Patients (2:1 male:female) were 35 to 75 years old (mean 56 ± 10), with T2DM for less than 10 years (mean duration 5.9 ± 3.3), and were free of major complications. Patients had baseline HbA1 c levels between 7.0% and 11% (mean entry HbA1 c 8.5% ± 0.9%). Observations in 48 patients who completed the initial 4-week treatment show a 0.6% reduction in HbA1 c. Body weight was reduced by ~2.3 kg (baseline weight 88.7 kg ± 7.1). Mild nausea and vomiting were the primary AEs observed in some patients.
**Conclusion:** Preliminary results after four weeks of daily CJC-1131 monotherapy treatment show clinically significant HbA1 c and body weight reductions, in patients with T2DM. At completion, the study will provide an indication of safety and efficacy after 3 months of CJC-1131 administration. These interim results confirm the efficacy and demonstrate the feasibility of using DAC technology to develop a GLP-1-based therapy for the treatment of patients with type 2 diabetes.

## 781

**Daily administration of GIP receptor antagonist alleviates glucose intolerance and counters insulin resistance in an animal model of obesity and Type 2 diabetes**

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**Background and aims:** Gastric inhibitory polypeptide (GIP) is a 42 amino acid gastrointestinal hormone secreted from endocrine K-cells in response to nutrient absorption. (Pro$^3$)GIP is a newly developed enzymatically stable and specific GIP receptor antagonist. The present study utilised (Pro$^3$)GIP to evaluate the effects of long-term ablation of endogenous GIP action on spontaneous obesity and diabetes using *ob/ob* mice.
**Materials and methods:** (Pro$^3$)GIP was synthesised using solid-phase Fmoc peptide chemistry and structure was confirmed by matrix assisted laser desorption mass spectrometry. Obese-diabetic (*ob/ob*) mice (12–14 weeks) were divided into two groups (n=6) that received, once daily i.p. injections (17.00 h) over an 11-day period, of either saline vehicle (0.9%, w/v, NaCl) or (Pro$^3$)GIP (25 nmol/kg body weight). In the subsequent 9 days, treatment was ceased. Food intake and body weight were recorded five days before and daily throughout the 20 day study period. Plasma glucose and insulin concentrations were measured at 3–4 day intervals. At the end of the treatment period (day 11), glycated haemoglobin (%$HbA_{1c}$), glucose tolerance (18 mmol/kg glucose i.p.) and insulin sensitivity (50 units insulin/kg bodyweight) of both groups were assessed. These tests were repeated on day 20 of the study after discontinuation of (Pro$^3$)GIP.
**Results:** Average body weights and food intakes did not significantly differ (P>0.05) between groups during the study. However, on day 11 of treatment, non-fasting plasma glucose levels were significantly (P<0.05) reduced in *ob/ob* mice receiving (Pro$^3$)GIP treatment (12.4 ± 0.8 mmol/l *vs.* 17.4 ± 1.3 mmol/l for control). Following i.p. glucose administration, the overall plasma glucose excursion was significantly (1.6-fold; P<0.05) lowered in the 11-day (Pro$^3$)GIP treated mice (220.5 ± 37.4 mmol/l.min *vs.* 342.7 ± 49.4 mmol/l.min for control). Plasma insulin concentrations were also significantly diminished 15, 30 and 60 min post glucose injection compared to controls (27%, 29% and 23%, respectively; P<0.05, P<0.01, and P<0.05). No significant difference in overall glucose-mediated insulin release was observed between the (Pro$^3$)GIP treated and control mice. Exogenous insulin injection caused a significantly greater fall of glucose concentrations in (Pro$^3$)GIP treated *ob/ob* mice (1828.5 ± 163.6 mmol/l.min *vs.* 1165.0 ± 199.1 mmol/l.min for control, P<0.05) indicative of substantial alleviation of insulin resistance. In keeping with improved glycaemic control, (Pro$^3$)GIP treated mice had significantly (1.2-fold, P<0.05) decreased %$HbA_{1c}$ (6.85 ± 0.38%) levels compared to controls (7.98 ± 0.36%). On day 20, after discontinuation of (Pro$^3$)GIP treatment, glucose and insulin concentrations, $HbA_{1c}$, glucose tolerance and insulin sensitivity were not significantly different from control *ob/ob* mice, indicating a reversal of the beneficial effects of (Pro$^3$)GIP administration.
**Conclusion:** These studies highlight GIP as an important link between obesity, insulin resistance and glucose intolerance. Once daily administration of the novel GIP receptor antagonist, (Pro$^3$)GIP, improved metabolic regulation in *ob/ob* mice, illustrating the potential of ablation of GIP receptor signaling for the treatment of obesity-related type 2 diabetes.

## 782

**Patients with Type 1 diabetes: perceptions associated with pramlintide as an adjunctive treatment to insulin**

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**Background and aims:** Pramlintide (PRAM) is an amylin analog studied as an adjunct to insulin in patients with diabetes.
**Materials and methods:** In a 29-week, randomized, triple-blind, placebo (PBO)-controlled trial in patients with type 1 diabetes, treatment satisfaction associated with PRAM was assessed in a study-specific, prospective, non-validated 14-item satisfaction survey using 6-point Likert-Scale questions ("strongly disagree" [1] to "strongly agree" [6]) at study exit. Of the 296 patients randomized, 266 patients who received PRAM 30 or 60 μg TID/QID in addition to insulin (CSII or MDI) completed the survey (age 41 ± 13 y, $HbA_{1c}$ 8.1 ± 0.8%, BMI 28 ± 5 kg/m$^2$).
**Results:** PRAM-treated subjects perceived greater beneficial effects on glucose, weight, and appetite control ($P<0.001$), irrespective of CSII or MDI regimen. They also reported significant improvements in their ability to function at home, work or school, how they felt overall, confidence in self management (all $P\leq0.001$), and reduction in "some worries" about having diabetes ($P=0.003$). PRAM-treated subjects, however, were aware of more side effects ($P=0.002$), but the overall score was relatively low (2.26 ± 1.64 [PRAM] vs. 1.74 ± 1.29 [PBO]). Subjects perceived that the benefits of PRAM outweighed the need for additional injections ($P<0.001$). Analyses of variance showed limited interaction between treatment and other covariates (age, gender, duration of diabetes, $HbA_{1c}$, and BMI) in the survey outcome, but subjects on CSII reacted more negatively to PBO treatment than those on MDI. Factor analysis showed that quality of life had an eigenvalue of 7.63 (54.5% of variance), and future use had an eigenvalue of 1.59 (11.4% of variance). The consistency of the survey, as assessed by Cronbach's alpha, was 0.93 for the scale as a whole, 0.94 for quality of life factor, and 0.69 future use factor.
**Conclusion:** In summary, these findings from a prospective, non-validated survey indicate favorable overall patient satisfaction with PRAM treatment and warrant further evaluation.

## 783

**Effects of pramlintide on the magnitude and speed of postprandial blood glucose fluctuations in patients with Type 1 diabetes**

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**Background and aims:** The magnitude and rate of blood glucose (BG) fluctuations are key characteristics of diabetes, predicting risks of significant hypoglycemia or hyperglycemia during the postprandial (PP) period. This retrospective analysis assessed the effects of pramlintide (PRAM) as adjunct to insulin therapy on the magnitude and rate of BG fluctuations in patients with type 1 diabetes mellitus (T1DM).
**Materials and methods:** In this 29-wk, randomized, triple-blind, placebo (PBO)-controlled trial, 296 patients were randomized to PRAM or PBO (TID or QID), and PRAM was escalated from 15 to 60 μg, as tolerated, during a 4-wk initiation period. This was followed by a 25-wk maintenance period (30 or 60 μg PRAM), with insulin doses optimized to achieve glucose targets. In this retrospective analysis of self-monitored BG records, 248 patients had the minimum number of readings required for analysis of preprandial and PP mean BG, low and high BG indices, pre-to-PP BG rate of change, and BG rate of change from PP to preprandial-next-meal.
**Results:** The PRAM group had a significant reduction in pre-to-PP rate of BG change ($F=81.2$, $P<0.0001$). The rate of BG change from PP to preprandial-next-meal was ~2-fold lower in the PRAM group, ($F=65.3$, $P<0.0001$), compared to PBO. Also, the PRAM group had a significantly lower mean PP BG value (8.4 mmol/L) than PBO (9.7 mmol/L; $F=81.2$, $P<0.0001$) and significantly lower risk for PP hyperglycemia ($F=71.3$, $P<0.0001$) compared to PBO. Significant differences were observed among the meal-related BG readings across the day with the hyperglycemia highest after breakfast in PBO, while in PRAM, PP BG did not vary after other meals throughout the day. The significant ($P<0.0001$) reduction in PP hyperglycemia in PRAM was achieved without an increased risk of preprandial hypoglycemia.
**Conclusion:** Thus, PRAM reduced the magnitude and rate of PP BG fluctuations in patients with T1DM, resulting in reduced PP risk for hyperglycemia without increased premeal risk for hypoglycemia.

## 784

**Assessing glucose variability using CGMS in pramlintide- and placebo-treated subjects with Type 1 diabetes mellitus**

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**Background and aims:** This retrospective analysis assessed the effect of pramlintide (PRAM) as an adjunct to insulin therapy on blood glucose (BG) fluctuations in subjects with type 1 diabetes mellitus (T1DM) using a continuous glucose monitoring system (CGMS) from Medtronic MiniMed.
**Materials and methods:** In a double-blind study, 24 subjects (22 evaluable) with T1DM (age 42 ± 11 y, $HbA_{1c}$ 8.2 ± 1.7%, mean±SD) using CSII with either lispro (n=21) or regular (n=3) insulin were randomized to preprandial injections of placebo (PBO) (n=6) or 30 μg PRAM TID (n=18) for 4 wks. Preprandial insulin doses were initially reduced by 10% to 20%. This retrospective analysis included 24-h CGMS profiles for 22 subjects (PRAM, n=16; PBO, n=6) who completed assessments at: baseline, 4 wks of study medication, and a 2-wk washout period. BG values were aggregated within 1-h blocks across the 24-h assessment period to overcome imprecision inherent with the CGMS curve fitting software. The primary analysis was BG rate of change, a parameter that estimates temporal fluctuations in BG level and has also been shown to predict acute changes in mood and cognitive symptoms.
**Results:** The BG rate of change (mean±SD) was significantly reduced following 4 wks of PRAM compared to PBO (26.4 ± 6.8 vs. 36.8 ± 11.1 mg/dL/h, $F=14.7$, $P<0.001$). The groups did not differ at baseline or after the 2-wk washout period. Other measures of variability such as SD of BG did not achieve statistical significance. However, secondary analyses performed *within* the 1-h blocks for each 24-h period demonstrated significant reductions in SD of BG ($F=8.8$, $P=0.005$) and BG range ($F=9.0$, $P=0.004$) in the PRAM vs. PBO group. Hypoglycemia risk trended lower in the PRAM group as measured by the low BG index ($F=4.04$, $P=0.058$).
**Conclusion:** In summary, this retrospective analysis of CGMS data indicates that PRAM treatment in subjects with T1DM reduced glucose fluctuations, especially BG rate of change, without increasing the risk of low BG values.

## 785

**Effect of pramlintide on ad-libitum food intake in obese subjects and subjects with Type 2 diabetes: a randomized, double-blind, placebo-controlled, crossover study**

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**Background and aims:** Long-term pivotal trials in insulin-treated subjects with type 2 diabetes showed that adjunctive treatment with the amylin analog pramlintide (PRAM) reduced $HbA_{1c}$ in conjunction with dose-dependent weight loss. Although amylin has been shown to reduce food intake in rodents, the effect of PRAM on food intake in humans has not yet been assessed.
**Materials and methods:** In this single-center, double-blind, placebo (PBO)-controlled, crossover study, 11 insulin-treated men with type 2 diabetes (T2DM: age 60 ± 3 y, BMI 28.9 ± 1.4 kg/m$^2$, $HbA_{1c}$ 7.8 ± 0.1%, mean±SE) and 15 non-diabetic obese men (OB: age 41 ± 5 y, BMI 34.4 ± 1.2 kg/m$^2$) underwent a standardized buffet meal test on two occasions. After an overnight fast, subjects received, in randomized order, a single subcutaneous injection of either PRAM (120 μg) or PBO, immediately followed by ingestion of a standardized, liquid preload meal (t=0 min). After 1 h, subjects were offered an ad-libitum buffet meal, with measurement of total energy and macronutrient intake, as well as meal duration.
**Results:** In all 26 subjects, energy intake at the buffet meal was reduced by 19 ± 5% with PRAM compared to PBO (818 ± 73 vs. 1002 ± 62 kcal, Δ −184 ± 47 kcal, $P=0.0004$, mean±SE). The reduction in energy intake with PRAM reflected equivalent reductions in fat (−19 ± 5%), carbohydrate (−17 ± 5%), and protein (−18 ± 5%) intake (all $P<0.005$ vs. PBO), occurred without a change in meal duration (27 ± 2 vs. 27 ± 2 min, n.s.), and was evident in both the T2DM (Δ −202 ± 64 kcal, −23 ± 8%, $P=0.017$) and OB (Δ −170 ± 68 kcal, −16 ± 6%, $P=0.013$) groups. In terms of gastrointestinal adverse events, 1 subject who received PRAM in the T2DM group reported nausea and anorexia.

**Conclusion:** These results suggest that reduced food intake may be a mechanism for the reduction in body weight observed with PRAM in long-term trials in insulin-treated patients with diabetes.

# PS 69

# DPP IV Inhibitors

## 786

**Reduced increments in total glucagon like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP) in plasma after a single dose of the dipetidyl peptidase-4 inhibitor LAF237 before an oral glucose load in healthy subjects**

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**Background and aims:** Inhibition of dipeptidyl peptidase-4 (DPP-4) reduces proteolytic degradation of the biologically active insulinotropic gut hormones glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), which are secreted in response to an oral glucose load. This has been reported to result in a higher proportion of structurally intact, biologically active hormones, potentially approaching 100 % of the GIP and GLP-1 present. In the present study the question was asked, how DPP-4 inhibition using LAF237 affects endogenous secretion of total GLP-1 and GIP after oral glucose loads in healthy subjects and how LAF237 interacts with the sulfonylurea glibenclamide to stimulate insulin secretion and to cause reactive hypoglycaemia.
**Materials and methods:** 16 healthy male subjects (age 29 ± 10 y., BMI 25.3 ± 2.8 kg/m$^2$, fasting plasma glucose 4.5 ± 0.3 mM, 2 h after oral glucose 4.5 ± 0.8 mM) received, on four separate days after an overnight fast, in random order, a single dose of study medication (LAF237 (L) [100 mg]) or placebo (P), with and without glibenclamide (G) (5 mg) in a double-blind, 4-way crossover study design. The study medication was given 30 min prior to 75 g oral glucose and blood was sampled for 6 hours to measure glucose, insulin, and total GLP-1 and GIP (RIA). Statistical evaluation was done using ANOVA.
**Results:** The positive integrated incremental responses of total GLP-1 were reduced by LAF237 by 72 % (P: 1577 ± 169 vs. L: 439 ± 169 pmol · l$^{-1}$ · min, LS mean ± SE) without glibenclamide, and by 48% (G: 1559 ± 177 vs. LG: 808 ± 169 pmol · l$^{-1}$ · min) with glibenclamide. There was a significant effect of LAF237 ($p < 0.0001$), but not of glibenclamide ($p = 0.31$) on total GLP-1, without an interaction of the effects ($p = 0.26$). Similarly, positive integrated incremental responses of total GIP were reduced by LAF 237 by 26 % (P: 4637 ± 408 vs. L: 3448 ± 408 pmol · l$^{-1}$ · min) without glibenclamide and by 21% (G: 4148 ± 427 vs. LG: 3287 ± 408 pmol·l$^{-1}$ · l$^{-1}$ · min) with glibenclamide. The effect of LAF237 on total GIP was significant ($p = 0.017$), but not the effect of glibenclamide ($p = 0.44$). There was no interaction of the two effects ($p = 0.69$) on total GIP. Although glibenclamide-stimulated insulin secretion was significantly enhanced with LAF237 ($p = 0.01$), reactive hypoglycaemia (≤1.9 mM) provoked by glibenclamide was not accentuated by the simultaneous administration of LAF237 ($p = 0.25$).
**Conclusion:** Administration of the DPP-4 peptidase inhibitor LAF237 acutely reduces the increments in total GLP-1 and GIP in response to oral glucose in healthy subjects. This is possibly explained by negative feedback regulation involving the sensing of intact, biologically active GLP-1 and GIP concentrations. These results could point to mediators in addition to biologically active GLP-1 and GIP to explain the antidiabetic actions of DPP-4 inhibition. There could also be a lower threshold for insulinotropic actions of incretin hormones in hyperglycaemic type 2 diabetic patients. Similar experiments need to be done in patients with Type 2 diabetes and more physiological meal stimuli.

## 787

**LAF237 is a DPP-4 inhibitor that improves model-assessed β-cell function in drug-naïve patients with Type 2 diabetes**

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**Background and aims:** It was previously reported that LAF237 (LAF) inhibits the incretin-inactivating enzyme, dipeptidyl peptidase 4, increases plasma levels of intact GLP-1 and reduces fasting (FPG) and postprandial glucose levels in patients with type 2 diabetes (T2DM). Surprisingly, plasma insulin levels were not affected by LAF. The aim of this study was to assess

the influence of LAF *vs* placebo on β-cell function in patients with T2DM not previously treated with oral agents.
**Materials and methods:** Patients were treated with LAF (100 mg, bid, n = 9) or placebo (PBO, n = 11) for 28 days. Insulin secretion rate (ISR, calculated by deconvolution of C-peptide levels) and plasma levels of glucose, insulin, and the intact (N-terminally detected) form of both GIP and GLP-1 were measured during 24-hr sampling (comprising 3 standardized meals) before (Day -1) and after 28-day treatment. β-cell function was evaluated with a model that describes insulin secretion as a function of absolute glucose levels, the rate of change of glucose (derivative factor), and a potentiation factor.
**Results:** The mean age, BMI, $HbA_{1c}$ and FPG of participants were 45.0 y, 32.1 kg/m$^2$, 7.5% and 8.9 mM, respectively, and did not differ significantly between groups. As shown in Table 1A, plasma levels of intact GIP and GLP-1 (13.5-hr mean = AUC/time) more than doubled during treatment with LAF, whereas the incretin hormones did not change significantly in PBO-treated patients. In patients taking LAF, FPG and 24-hr mean glucose each decreased by 1.2 ± 0.4 mM relative to PBO ($P<0.05$). Neither fasting, nor 24-hr mean plasma insulin levels were significantly affected by LAF; however, as shown in Table 1B, LAF amplified the β-cell response to glucose. The ISR at each glucose level calculated was significantly higher in LAF *vs* PBO-treated patients, but the β-cell sensitivity to glucose (SLM7, slope of glucose dose/response between 7 and 9 mM) was not significantly affected by LAF (95% CI: [-19.5%, +99.1%]). The LAF increase in the secretory tone defined as ISR @7–9 mM may explain at least in part, the LAF induced decrease in FPG and 24-hr mean glucose levels. LAF did not influence the model-derived potentiation or derivative factors.

| | LAF237 (n = 9) | | Placebo (n = 11) | | LAF *vs* PBO[1] |
|---|---|---|---|---|---|
| A: 13.5-hr mean | Day -1 | Day 28 | Day -1 | Day 28 | *P* value |
| GIP (pM) | 41.0 ± 5.5 | 94.0 ± 12.1 | 33.7 ± 8.0 | 42.4 ± 8.5 | < 0.001 |
| GLP-1 (pM) | 7.7 ± 2.8 | 18.5 ± 3.4 | 7.4 ± 1.7 | 7.4 ± 1.7 | < 0.001 |
| **B: ISR** (pmol/min/m$^2$) | Day -1 | Day 28 | Day -1 | Day 28 | |
| ISR @ 7 mM | 283 ± 71 | 328 ± 77 | 228 ± 38 | 184 ± 29 | < 0.01 |
| ISR @ 9 mM | 460 ± 122 | 530 ± 142 | 363 ± 72 | 284 ± 47 | < 0.05 |
| SLM7 | 88 ± 26 | 101 ± 33 | 67 ± 18 | 50 ± 9 | NS |

[1]Log-transformed ANCOVA
Excluding one outlying PBO-treated patient, ISR7 increased with intact GLP-1 (N = 19, $P = 0.043$, $r^2 = 0.22$, log-log scale), consistent with prior published work.
**Conclusion:** By increasing the active form of GLP-1 and/or GIP, LAF237 improves β-cell function via enhanced secretory tone.

## 788

### A novel, orally active dipeptidyl peptidase IV inhibitor potently improves glucose tolerance in mice

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**Background and Aims:** The potential of DPP-IV inhibitors as treatment for diabetes is primarily due to their ability to increase GLP-1 and insulin levels only in the presence of a glucose load. The present study describes the biological profile of a novel DPP-IV inhibitor GRC-8087.
**Materials and Methods:** DPP-IV activity was determined using a fluorescence-based assay by the cleavage rate of 7-amino-4-methyl coumarin (AMC) from synthetic substrate Glycyl-Prolyl-AMC using human or rat enzyme. Lean, 18 h-fasted male C57BL/6J mice (19–25 g) received an oral glucose load of 2 g/kg followed immediately by either vehicle or different doses of GRC-8087. Blood samples were collected from tail at 0, 30, 60, 90 and 120 mins after administration and blood glucose was measured using Glucotide strips. Pharmacokinetic studies were performed on Sprague Dawley rats of either sex weighing between 150–180 gms.
**Results:** GRC-8087 showed an IC50 of 13.9 nM against human recombinant DPP-IV and IC50 s of 26.5 nM, 9.7 nM and 16.3 nM in human plasma, CaCo-2 and rat plasma systems respectively. Kinetic studies revealed that GRC-8087 is a competitive and reversible inhibitor of the DPP-IV enzyme. In specificity studies, GRC-8087 had IC50's of 6.4 μM against DPP-II (450-fold) and 3.5 μM against PPCE (250-fold), while at 1 mM it did not produce inhibition of Leu-aminopeptidase, trypsin, chymotrypsin and elastase. GRC-8087, at doses of 0.3, 1, 3 and 10 mg/kg, po, produced significant mean percent reductions of 21.7, 25.0, 33.6 and 44.6 ($p<0.05$ for all doses tested) respectively in areas under the glucose tolerance curve. GRC-8087 also exhibited rapid absorption with an absolute oral bioavailability of 100 %, showing peak concentration of 1.45 μg/ml at nearly 0.2 h and a half life of 0.54 h. It has a clearance of 20 L/hr/kg and a volume of distribution of 3.4 L/kg on i.v. administration.
**Conclusion:** GRC-8087 is a potent, selective, rapidly absorbed, orally active inhibitor of human and rodent DPP-IV.

## 789

### Plasma DPP-IV activity is increased in Type 2 diabetic patients in the fasting state

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**Background and aims:** Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are incretin hormones, secreted in response to meal ingestion. The incretin hormones stimulate insulin secretion and are essential for maintenance of normal blood glucose concentrations. Type 2 diabetic patients have an impaired incretin effect. Both incretin hormones are metabolized quickly by the enzyme dipeptidyl peptidase IV (DPP-IV). Therefore, the aim of the present study was to investigate plasma DPP-IV activity in the fasting and the postprandial state in type 2 diabetic patients and healthy subjects.
**Materials and methods:** The study was divided into two protocols. Protocol one; 40 fasting type 2 diabetic patients (28 men); age: 61 (36–78) years, BMI: 31 (25–41) kg/m$^2$, HbA1 c: 8.6 (4.7–12.4) % and 20 healthy matched subjects, Protocol two; Eight type 2 diabetic patients (6 men) age: 63 (57–67) years, BMI: 33 (31–35) kg/m$^2$, HbA1 c: 7.5 (6.3–9.8) % and 8 healthy matched subjects. In protocol one, fasting values of DPP-IV activity were evaluated and in protocol two, postprandial DPP-IV activity during a standard meal tests (566 kcal) was evaluated.
**Results:** Mean fasting plasma DPP-IV activity was significantly higher in the patient group compared to the healthy subjects (67.5 ± 1.9 % vs. 56.8 ± 2.2 % (mean±SEM); p=0.001). In the type 2 diabetic patients, a positive correlation was seen between DPP-IV activity and FPG, $HbA_{1c}$. Negative correlation was seen between plasma DPP-IV activity and duration of the diabetes as well as the age of the patients. No postprandial changes were seen in plasma DPP-IV activity in any of the groups.
**Conclusion:** The present data show that plasma DPP-IV activity level is raised in type 2 diabetic patients in the fasting state compared to healthy subjects. This may contribute to the lower postprandial concentrations of intact GLP-1 seen in type 2 diabetic patients and thereby contribute to the impaired incretin effect seen in type 2 diabetics. Plasma DPP-IV activity does not seem to be regulated by meal ingestion.

## 790

### Dipeptidyl-Peptidase IV (DPPIV) activity and metabolic control in Type 2 diabetes

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**Background and aims:** We have recently reported that chronic hyperglycaemia increases DPPIV activity in human endothelial cells in vitro; considering that DPPIV inactivates the insulinotropic hormone Glucagon-Like Peptide-1(GLP-1), a hyperglycaemia-induced increase of DPPIV could contribute to the previously reported impairment of post-prandial GLP-1 response in type 2 diabetes.
**Materials and methods:** We compared plasma DPP-IV activity in a sample of 32 type 2 diabetic outpatients with HbA1 c>7.5%, aged 67.9 ± 9.3 y, with BMI 29.9 ± 5.5 kg/m$^2$ (Sample A) with age-, sex-, and BMI-matched samples of newly-diagnosed type 2 diabetic subjects with HbA1 c<6.5% (B), subjects with impaired (C) and normal (D) glucose tolerance. A further sample of 66 type 2 diabetic patients aged 61.8 ± 13.7 years, with BMI of 29.0 ± 5.9 kg/m$^2$, and HbA1 c of 7.6 ± 1.3% was observed prospectively for three months, measuring HbA1 c and DPPIV.
**Results:** A significantly ($p<0.05$) higher DPPIV activity was measured in sample A (27.12 ± 7.1 U/l) than in samples B (22.2 ± 6.0), C (18.8 ± 6.8) and D (21.1 ± 7.0); in sample A, DPPIV showed a significant correlation with fasting glucose (r=0.42). Baseline DPPIV in the further sample of 66 type 2

diabetic patients showed a significant correlation with HbA1 c (r= 0.25; $P<0.05$), and 90-day variations of DPPIV showed a significant correlation with those of HbA1 c (r=0.26; $P<0.05$).
**Conclusion:** In conclusion, type 2 diabetic patients in good metabolic control show a DPPIV activity similar to that of matched controls; a higher degree of hyperglycaemia is associated to increased DPPIV activity, which could contribute to impairment of GLP-1 response to meals.

## 791

**The novel DPPIV inhibitors NN7201 and LAF237 acutely and chronically improve glucose tolerance in GK rats, but do not have any beneficial effects on diurnal glycaemia or $HbA_{1C}$**

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**Background and aims:** Preclinical and clinical studies have suggested that DPPIV inhibitors are efficacious in treating the hyperglycaemia of type 2 diabetes. NN7201 (NN), a xanthine based DPPIV inhibitor, and LAF237 (LAF), were compared with respect to their ability to lower $HbA_{1C}$ in the GK rat, a model of modest type 2 diabetes.
**Materials and methods:** 9 week (W) old GK rats were allocated into 3 treatment groups (N=10) with the same mean $HbA_{1C}$, orally dosed with 10 mg/kg NN, LAF or vehicle (VE) twice daily for 8W. In conjunction with the first dose and after 3 and 8W of dosing, $HbA_{1C}$ and glucose tolerance (OGTT) were determined, and after 4W, an 18 hour (H) profile of BG was measured. DPPIV activity was measured before, and after 3W of dosing. Food intake (FI) and body weight (BW) were monitored throughout the study. Data are expressed as mean ± SEM.
**Results:** During the first OGTT, a significant and comparable reduction in $AUC_{GLU}$ was seen with NN (320 ± 24 mM * min, $p<0.001$) and LAF (355 ± 31 mM * min, $p<0.01$), as compared to VE (583 ± 32 mM * min). Similar data on OGTT was obtained after 3 and 8W of treatment. Mean BG levels during the 18H profile after 4W of treatment were not significantly different in VE treated rats (6.82 ± 0.22 mM) as compared to NN (6.69 ± 0.18 mM) or LAF (6.86 ± 0.21 mM) treated. After 8W of treatment, $HbA_{1C}$ was not significantly decreased in either NN (3.70 ± 0.05) or LAF (3.72 ± 0.02), as compared to VE (3.80 ± 0.04) treated, rats. DPPIV activity in the plasma of VE treated rats was measured to be 37 ± 4%, whilst there was no detectable activity in NN or LAF treated rats. No differences were seen in FI or BW between the treatment groups.
**Conclusion:** Inhibition of DPPIV acutely and chronically improves glucose tolerance in GK rats although this does not translate into improvement of diurnal glycaemia or $HbA_{1C}$. Future studies should address whether the lack of effects on $HbA_{1C}$ are related to treatment principle or to the fact that postprandial glycaemia is not a major cause of elevated $HbA_{1C}$ in GK rats.

## 792

**Decreased dipeptidyl peptidase-IV activity and degradation of glucagon-like peptide-1(7-36)amide in Type 2 diabetes**

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**Background and aims:** Dipeptidyl peptidase IV (DPP IV) is a ubiquitous enzyme that plays a key role in degradation and metabolic inactivation of glucagon-like peptide-1 (GLP-1) by removal of the N-terminal dipeptide $His^7$-$Ala^8$. In view of possible use of GLP-1 analogues and/or DPP IV inhibitors for diabetes therapy, the present study examined DPP IV activity in type 2 diabetic subjects with regard to metabolic control and GLP-1 degradation
**Materials and methods:** Mid-morning blood samples were collected from controls and from type 2 diabetic subjects under good ($HbA_{1c}$ <7%), moderate ($HbA_{1c}$ 7–9%) and poor glycaemic control ($HbA_{1c}$ >9%). All samples were analysed for DPP IV activity using a fluorimetric assay system involving the substrate Gly-Pro-aminomethylcoumarin with a standard curve of aminomethycoumarin (AMC, concentration range 0–15 nmol/tube) measured at excitation λ 370 nm, emission λ 440 nm. One unit of DPP IV activity was defined as the enzyme activity that produced 1 nmol of AMC in 10 µl of plasma per minute. Other parameters measured included the percentage $HbA_{1c}$, plasma glucose, insulin and C-peptide concentrations. Stability of intact GLP-1 (5 µg) was determined in the human control and diabetic plasma samples (10 µl, n=3) at 0, 2, 4 and 8 h at 37°C. Peptide degradation was quantified by reversed-phase HPLC analysis on a Vydac C-18 analytical column (4.6x250 mm), with structural confirmation by matrix assisted laser desorption mass spectrometry.
**Results:** The mean circulating activity of DPP IV in healthy control subjects ($HbA_{1c}$ 5.9 ± 0.01%, glucose 5.5 ± 0.2 mmol/l, insulin 181.2 ± 24.0 pmol/l) was 22.5 ± 0.7 nmol/ml/min (n=70). In type 2 diabetic subjects, the circulating DPP IV activity ($HbA_{1c}$ 7.6 ± 0.2% ($P<0.001$), glucose 10.6 ± 0.6 mmol/l ($P<0.001$), insulin 212.4 ± 4.4 pmol/l) was significantly decreased at 18.1 ± 0.7 nmol/ml/min ($P<0.001$, n=54). DPP IV was reduced 1.2-fold ($P<0.01$, n=25), 1.3-fold ($P<0.001$, n=19) and 1.3-fold ($P<0.05$, n=10) within the good, moderate and poorly controlled diabetic groups, corresponding to 18.7 ± 1.0, 17.4 ± 1.4 and 18.0 ± 1.5 nmol/ml/min of DPP IV, respectively. In these 3 diabetic groups $HbA_{1c}$ concentrations were 6.4 ± 0.1% ($P<0.001$), 7.9 ± 0.1% ($P<0.001$), 9.9 ± 0.2% ($P<0.001$), plasma glucose concentrations were 8.4 ± 0.4 ($P<0.001$), 11.4 ± 0.8 ($P<0.001$) and 14.4 ± 1.1 ($P<0.001$) mmol/l, and mean insulin concentrations were 41.5 ± 7.6, 27.5 ± 3.7, and 34.3 ± 12.0 mU/l, respectively. Intact GLP-1 present in plasma at t=0, 2, 4, 8 h in healthy control subjects was 100 ± 0.0%, 65 ± 2.0%, 41.5 ± 3.5%, 27.5 ± 2.5% and in type 2 diabetic subjects was significantly greater at 100 ± 0.0%, 74 ± 0.0% ($P<0.05$), 57 ± 2.0%, 40 ± 2.0% ($P<0.05$), respectively.
**Conclusion:** Plasma DPP IV activity and resultant GLP-1 degradation is reduced in diabetes, possibly reflecting an adaptive negative effect on DPP IV gene expression. Decreased enzyme activity may contribute to altered beta cell responsiveness in diabetes and facilitate the use of GLP-1 analogues in type 2 diabetes therapy.

# PS 70

# Novel oral agents

## 793

**The novel, xanthine-containing, DPP-IV inhibitor NNC 72-2138 improves glucose tolerance in Zucker obese male rats**

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**Background and aims:** The naturally occurring protease DPP-IV promotes rapid degradation of circulating GLP-1. This study compares the effects of NNC 72-2138 (NNC) with LAF 237 (LAF) and a prototypal DPPIV inhibitor, valine pyrrolidide (VP), in Zucker obese (ZO) rats.
**Materials and methods:** ZO male rats (8 weeks old) were allocated into 4 groups having matched response to an oral glucose tolerance test (OGTT, 2 g/kg). Rats were given either vehicle (VEH), NNC (10 mg/kg), LAF (10 mg/kg) or VP (30 mg/kg) po twice daily. On the 1st, 7th and 14th day of treatment an OGTT was performed. On day 14, a large blood sample was obtained for measurements of total and intact GLP-1 and DPP-IV activity. Food and body weight were monitored weekly.
**Results:** Data are shown in the table as mean±SEM (n=6/group). ANOVA incl.Tukey's post hoc test.*P<0.05 versus vehicle. BLD: below detection limit of assay. There were no differences in food intake and body weight between the groups.
**Conclusion:** Inhibition of DPP-IV both acutely and chronically improves OGTT in insulin resistant Zucker rats. The data support the potential of DPP-IV inhibitors as therapeutic agents for the treatment of impaired glucose tolerance and type 2 diabetes

Results

| | Day 1 $OGTT_{AUC}$ (mMxmin) | Day 7 $OGTT_{AUC}$ (mMxmin) | Day 14 $OGTT_{AUC}$ (mMxmin) | DPP-IV activity (%) | GLP-1 Total (pM) | GLP-1 Intact (pM) |
|---|---|---|---|---|---|---|
| VEH | 797 ± 27 | 867 ± 46 | 988 ± 58 | 32.5 ± 2.7 | 143 ± 19 | 15.1 ± 1.1 |
| VP | 683 ± 12* | 675 ± 18* | 738 ± 13* | 2.5 ± 1.5* | 46 ± 7* | 21.6 ± 3.3 |
| LAF | 651 ± 8* | 705 ± 19* | 799 ± 49* | BDL* | 73 ± 22* | 28.8 ± 1.1* |
| NNC | 685 ± 28* | 735 ± 26* | 846 ± 41* | BDL* | 82 ± 11 | 29.4 ± 1.8* |

## 794

**The DP-IV inhibitor MK-0431 enhances active GLP-1 and reduces glucose following an OGTT in Type 2 diabetics**

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**Background:** MK-0431 is an orally active, potent, and highly selective dipeptidyl-peptidase (DP-IV) inhibitor being developed for the treatment of type 2 diabetes (T2D). DP-IV inhibitors are a new therapeutic approach to T2D that enhance levels of the active form of incretins, facilitating glucose-dependent insulin secretion.
**Material and methods:** A randomized, placebo-controlled, 3-period, crossover study was conducted in 56 patients with T2D on diet exercise treatment to assess the glucose-lowering activity as well as safety and tolerability of single oral doses of MK-0431. Patients received single oral doses of 25-mg or 200-mg MK-0431 or placebo, separated by 7-day washout intervals. Following an overnight fast, patients had an oral glucose tolerance test (OGTT) at 2 hours post dose.
**Results:** MK-0431 was generally well tolerated. MK-0431 was associated with a significant reduction of glycemic excursion following the OGTT: incremental glucose AUC was reduced by approximately 22% ($p<0.001$) and 26% ($p<0.001$), for the 25- and 200-mg doses respectively, as compared to placebo. Both doses were associated with approximately 2-fold increases in active GLP-1 levels as well as the ratio of active to total GLP-1 levels following an OGTT ($p<0.001$), as compared to placebo. Doses of 25-mg and 200-mg were associated with increases in plasma insulin AUC (22% and 23% respectively, $p<0.001$), plasma c-peptide AUC (13% and 21% respectively, $p<0.001$), and reductions in plasma glucagon AUC (8%, $p=0.015$ and 14%, $p<0.001$ respectively), following the OGTT, as compared to placebo.
**Conclusions:** The effects on glucose, active GLP-1, insulin, c-peptide and glucagon levels following the OGTT provide pharmacologic proof-of-concept for MK-0431 in patients with type 2 diabetes.

## 795

**Long term continuous application of the DP IV inhibitor P32/98 to rats increase plasma DP IV levels and effects islet morphology**

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**Background and aims:** Previous work revealed an increase of about 20% of plasma DP IV-activity after sub-chronic bid-administration of the DP IV-inhibitor P32/98 to VDF (fa/fa) Zucker Rats (Pospisilik et al, Diabetes 2002, 51, 943–950). Such positive feedback of DP IV-inhibition on DP IV-activity might impair long-term efficacy of drug therapy and cause also unexpected side-effects. Hence, we simulated long-term chronic DP IV-blockage by constant compound application to investigate the impact of sustaining daily DP IV-inhibition on DP IV-activity and on islet morphology as a biological readout.
**Materials and methods:** Male three-month-old Wistar / Furth rats (WF/Ztm) (n=30) were kept under SPF conditions. Basal fluid consumption per 24 h was monitored resulting in 12 ml / 24 h fluid consumption. Accordingly, drinking solutions adjusted for body weight (BW) with 0 mg/12 ml / kg(BW), 1 mg/12 ml / kg(BW), 10 mg/12 ml / kg(BW) of P32 / 98 (n=10 / group) were provided ad lib for a period of 7 weeks, respectively. Light protected drinking bottles were changed twice a week. After seven weeks animals were deeply anaesthetized (EDTA plasma collected), perfused transcardially with PFA according to standard protocols and brains, thymus, gaster, liver, and pancreas removed for further analysis. Islet morphology of the endocrine pancreas was morphometrically analyzed with respect to size, area and cellular composition. Plasma DP IV activity was measured in each group after 7 weeks of treatment using the flourescence substrate Gly-Pro-AMC
**Results:** After 7 weeks of continuous oral application of P32/98 via drinking water, plasma DP IV activity increased in the treated groups dose dependently from 8.9 ± 0.1 U/l in the control group to 17.4 ± 1.7 U/l and 25.4 ± 0.8 U/l in the 1 mg/kg and 10 mg/kg group, respectively. In the pancreas, the islets showed changes in size and islet and beta-cell area, while the cellular composition of the islets remained unchanged between the experimental groups. In the control group only small and medium-sized islet were found in the pancreas parenchyma. The morphometrical analysis revealed a dramatically increased islet area in the treated groups dose dependently from 9486 $\mu m^2$ in the control group to 11344 $\mu m^2$ and 16976 $\mu m^2$ in the 1 mg/kg and 10 mg/kg group, respectively.
**Conclusion:** Long-term continuous inhibition of DP IV may trigger a compensatory response from the body of increased DP IV production. Alternatively, the increased plasma activity may be a result of increased cell death and release of the cell bound DP IV to the plasma. Further investigations are necessary to study the cause for DP IV increase in more detail, but these results indicate that an antidiabetic treatment with stable DP IV inhibitors, which permanently block DP IV activity, may result in tachyphylaxis. The observed changes in the endocrine pancreas may be caused by changes in the cell cycle of pancreatic beta-cells.

## 796

**Fructose 1,6-bisphosphatase inhibition improves oral glucose tolerance and enhances the antidiabetic action of glyburide in the Zucker diabetic fatty rat**

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**Background and aims:** MB06322 is an oral prodrug of a novel agent (MB05032) that specifically inhibits a key rate-limiting enzyme of gluconeogenesis (GNG), fructose 1,6-bisphosphatase (FBPase). Enhanced flux through the GNG component of endogenous glucose output (EGP) is a common finding in patients with type 2 diabetes (T2DM) and has been proven to contribute significantly to fasting hyperglycemia. Impaired suppression of EGP in T2DM patients is an important contributor to postprandial hyperglycemia. However, the specific contribution of upregulated GNG to the latter abnormality is unknown. A primary purpose of these investigations was to assess the role of GNG in postprandial hyperglycemia through evaluation of MB06322 in an oral glucose tolerance (OGT) model in the Zucker Diabetic Fatty (ZDF) rat. The potential utility of MB06322 for the treatment of postprandial hyperglycemia as a monotherapy and in combination with glyburide, a widely prescribed insulin secretagogue of the sulfonylurea class, was also explored.
**Materials and methods:** Following a 4-hour fast, 8–10 week old ZDF rats were divided into four glucose-matched groups of n = 20–23: vehicle, MB06322, glyburide, or the combination of MB06322 and glyburide. Animals which did not meet the following baseline criteria after the 4-hour fast were excluded: blood glucose (BG) 250–500 mg/dL; plasma insulin > 2.5 ng/mL. Animals outside these ranges were found to respond poorly to glyburide. Previously established, maximally effective doses of MB06322 and glyburide (300 and 100 mg/kg, respectively) were administered orally (4.5 h into fast) and followed by an oral glucose load of 2 g/kg (6 hours into fast). Blood samples were taken prior to and intermittently for 3 hours following glucose administration.
**Results:** OGT was significantly improved following administration of either MB06322 or glyburide (ΔBG 60 and 105 mg/dL, respectively). Combination treatment was superior to either monotherapy, markedly blunting the BG excursion (ΔBG 170 mg/dL) and, by 2 hours post load, lowering BG to below baseline levels. MB06322 monotherapy did not stimulate insulin release, but was associated with a modest elevation of plasma lactate that tended to be attenuated in the combination-treated group. The insulin secretory response was identical in the glyburide monotherapy and combination groups. Glucose alone elicited no apparent stimulation of insulin secretion, indicative of advanced pancreatic dysfunction in the animals.
**Conclusion:** Both MB06322 and glyburide monotherapies improved OGT in the 6-hour fasted ZDF rat. The efficacy of MB06322 as a monotherapy suggests GNG is active in the postprandial state and is a significant contributor to postprandial hyperglycemia, a major abnormality associated with T2DM. Combination therapy was more effective than either therapy alone, supporting the potential utility of FBPase inhibitor/insulin secretagogue combination therapy for the treatment of T2DM patients.

## 797

***In Vivo* studies of a novel glucose lowering agent, ISF402**

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**Background and aims:** ISF402 is an analogue of a naturally occuring urinary tetrapeptide that reduces blood glucose in NZO rabbits. ISF402 may be a suitable treatment for insulin resistance and Type 2 Diabetes, however, little is known about its mode of action. The aims of this study was to use insulin-resistant Zucker rats as a model for Type 2 Diabetes to 1)identify an effective dose and assess the efficacy of ISF402 and 2)establish whether glucose lowering was due to effects on insulin secretion or improved insulin sensitivity.
**Materials and methods:** ISF402 was administered by intravenous (IV) or intraperitoneal (IP) injection to 16–20 week old female Zucker rats at doses of 1.5, 3, 4.5 and 10 mg/kg with or without co-injection of insulin at 1U/kg (IV) (n=50) or 2U/kg (IP) (n=16). Blood and serum were collected and blood glucose, insulin and C-peptide concentrations were measured by standard assays. An index of insulin sensitivity (IIS) was calculated from fasting blood glucose (FBG) and C-peptide (FCP) concentrations as follows: IIS= FBG × FCP.
**Results:** The Zucker rats were insulin resistant as shown by the small reduction in blood glucose during IV insulin tolerance tests. When ISF402 was co-injected with the insulin at 1.5 mg/kg and 3 mg/kg, blood glucose concentrations were reduced significantly (rmANOVA $p<0.001$ and $p=0.03$, respectively) compared to injection of insulin alone at 20–60 mins after administration. Administration of ISF402 at 4.5 mg/kg without exogenous insulin also reduced blood glucose concentrations at 30–60 mins after injection (rmANOVA $p=0.02$) compared to control group (Saline). A rapid increase in serum insulin concentrations was detected 10 mins after co-injection of ISF402 with insulin, but not after injection of either insulin or ISF402 alone. The increase in serum insulin was accompanied by a reduction in C-peptide concentration indicating that endogenous insulin secretion was reduced. Thus, ISF402 does not stimulate the release of endogenous insulin but instead reduces the clearance rate of the co-injected insulin. Similar results were observed for co-injection of ISF402 at 3 mg/kg with insulin by the IP route. IP injection of ISF402 at 3 mg/kg with insulin exhibited a reduction in blood glucose and increase in serum insulin. However, this occured 15 mins later and insulin and blood glucose changes were prolonged over the course of the insulin tolerance test. The long- term efficacy of ISF402 was tested in Zucker rats by weekly injection of ISF402. Assessment of fasting glucose and C-peptide occured 3 weeks later. The index of insulin sensitivity was significantly lower in treated animals than in saline- injected controls ($p=0.008$, Student's t-test) indicating a prolonged improvement in insulin sensitivity.
**Conclusion:** ISF402 significantly improves insulin tolerance in insulin resistant Zucker rats. Improved insulin tolerance may be due to a reduction in the rate of insulin clearance from the circulation thereby increasing the availability of insulin to peripheral tissues. However, injection of ISF402 alone was effective in lowering blood glucose with no coincident increase in serum insulin levels, suggesting that an insulin-sensitising effect also exists. This study shows that ISF402 may prove a useful treatment for hyperglycaemia and insulin resistance in patient with Type 2 Diabetes.
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## 798

**Microphysiometry studies of insulin sensitising factor 402 in $C_2C_{12}$ muscle cells**

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**Background and aims:** Insulin Sensitising Factor (ISF 402), is a synthetic analogue of a naturally occuring tetrapeptide that was originally purified from human urine. ISF 402 has been shown to lower blood glucose *in vivo* in rodent models of insulin resistance. In order to understand the actions of ISF 402, we have employed *in vitro* cell studies and microphysiometry to measure the rate at which cell excrete acid during basal or stimulated conditions. The aim of this study was to investigate cell surface receptor activation in $C_2C_{12}$ cells in response to insulin and ISF 402.
**Materials and methods:** $C_2C_{12}$ cells were cultured and differentiated on cell supports. During analysis in the cytosensor cells were incubated in low-buffered RPMI without serum and exposed to increasing concentrations of insulin or ISF 402. Cellular response was detected by changes in pH. At the conclusion of the experiments 57 mM potassium chloride was added to determine the maximal response as a measure of cell viability.
**Results:** Insulin and ISF 402 both stimulated $C_2C_{12}$ muscle cells and showed bell-shaped dose response curves. The cellular response to insulin increased with increasing insulin concentrations over the range $10^{-4}$ to 100 nM. However, high concentrations of insulin (above $10^2$ nM) decreased the cellular response. Similarly cellular response increased with increasing concentrations of ISF 402 in the range of $10^{-3}$ μM to 20 μM and decreased at concentrations above 50 μM. ISF 402 showed an apparent insulin sensitising effect since exposure of cells to increasing insulin concentrations with a constant concentration of ISF 402 reduced the dose of insulin at which the maximum cellular response occured. This suggests that there may be a common component to the insulin and ISF 402 signalling pathways. In further studies the Phosphoinositol-3-Kinase (PI-3-kinase) inhibitor wortmannin (50 nM) decreased the cellular response to both insulin and ISF 402 implicating PI-3-kinase activation in cellular activation by insulin and ISF 402.
**Conclusion:** We conclude that the glucose lowering activity of ISF 402 observed *in vivo* can be explained by insulin-like activity or insulin sensitisation mediated by PI-3-kinase. Since ISF 402 and insulin apear to share a common signalling pathway attention is now being directed at interactions between ISF 402, insulin and the insulin receptor.
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## 799

**Curcumin inhibits hepatic glucose production in mice**

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**Background and aims:** Curcumin is the major yellow pigment extracted from turmeric, the powdered rhizome of the herb curcuma longa. Curcumin is reported to have a wide range of therapeutic effects; it is anti-inflammatory, cancer-chemopreventive, choleretic, and anti-diabetic. As curcumin is known to act on the liver, the anti-diabetic effect may attribute to the inhibition of elevated hepatic glucose production, which is frequently reported in type 2 diabetes. In this study, we have examined the effects of curcumin on hepatic glucose production and analyzed the mechanism of action.
**Materials and methods:** Isolated hepatocytes of 18-hours-fasted C57/BL6J mice were obtained by collagenase digestion. The fresh hepatocytes were incubated for 2 hours at 37C in a humidified atmosphere (5% CO2) in 0.5 ml of DMEM without glucose but containing glucogenetic substrate of 1 mM pyruvate or 2 mM dihydroxyacetone phosphate, and 0.24 mM 3-isobutyl-1-methylxanthine in the presence of curucumin or vehicle. The neosynthesized glucose in the supernatant was measured by glucose oxidation method. For the glycogenolysis experiment, isolated hepatocytes of fed mice were used. The enzyme activity of glucose-6-phosphatase was measured photometrically using liver microsomal fraction. The enzyme activity of fructose-1, 6-bisphosphatase and phosphoenolpyruvate carboxykinase were measured using liver cytosolic fraction.
**Results:** After exposure to curcumin, the gluconeogenesis from pyruvate was significantly inhibited in a concentration-dependent manner with an EC50 value of 15 microM and a maximal inhibition rate of 50%. After exposure to curcumin, gluconeogenesis from dihydroxyacetone phosphate was also significantly inhibited by 35%. After exposure to curcumin, glycogenolysis was also significantly inhibited by 25%. Curcumin itself did not affect the viability of hepatocytes. After exposure to 100 microM curcumin, the activity of hepatic glucose-6-phosphatase was inhibited by 50%, but neither the enzyme activity of fructose-1, 6-bisphosphatase nor phosphoenolpyruvate carboxykinase was affected.
**Conclusion:** Curcumin inhibits hepatic gluconeogenesis and glycogenolysis by inhibiting the activity of glucose-6-phosphatase, leading to the inhibition of hepatic glucose production. Thus, curcumin may provide a useful new therapy in treatment type 2 diabetes.

## 800

**Evidence for insulin independent suppression of glucagon secretion by LAF237**

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**Background:** LAF237 is a dipeptidyl peptidase 4 inhibitor which, results in the potentiation of the incretin hormones GLP-1 and GIP. It was previously reported that treatment with LAF237 leads to a 21% suppression of postprandial glucagon secretion in type 2 diabetes mellitus (T2DM), but whether this is attributable to direct endocrine effects of GLP-1 on glucagon or is a paracrine effect due to stimulation of insulin secretion is unclear. In order to address this question, we studied patients with type 1 diabetes mellitus (T1DM), thereby removing influence of endogenous insulin secretion.
**Materials and Methods:** This was a randomized, placebo-controlled, double-blinded crossover trial in twelve insulin-pump treated patients (eight females and four males) with T1DM; eleven participants completed the study. Participants received placebo or LAF237 100 mg bid for 28 days, followed by standardized meals (6 kcal/kg with 50% carbohydrate, 30% fat and 20% protein), with 6.5 hr blood sampling at the end of each 28-day treatment. Pre-prandial insulin boluses were similar (3.9 ± 2 U/kg) and basal rates of pump delivery ( 0.9 ± 0.4 U/kg ) were identical during LAF237 and placebo treatment.
**Results:** Fasting glucagon plasma levels did not differ following 28 days of placebo and LAF237 administration. Postprandial glucagon increased following meal ingestion, rising to a peak of 85 pg/mL at 60 minutes during placebo treatment, gradually returning to basal values of 60 pg/mL by the 5th hour. When compared to placebo, LAF237 treatment reduced the postprandial glucagon exposure by 12% (18.8 pg/mL*hr) during the first 2 hours ($AUC_{0-120\,min}$) (p=0.05) and by 10% overall ($AUC_{0-300\,min}$).
**Conclusions:** LAF237 suppressed prandial glucagon secretion in patients with T1DM treated by insulin pump therapy . These findings provide evidence that the glucagonostatic effect of the GLP-1 potentiating agent LAF237 is mediated via an endocrine effect rather than by a paracrine effect dependent on endogenous insulin release.

# PS 71

# Alternative oral and nutritional agents

## 801

**Coffee consumption and the likelihood of acute coronary syndromes in diabetic subjects: the CARDIO 2000 II Study**

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**Background and aims:** The effect of coffee consumption on the cardiovascular system has been debated for many years. In this case-control study we evaluated the association between various quantities and qualities of coffee drinking and the development of acute coronary syndromes, among diabetic subjects.
**Material and methods:** We studied demographic, lifestyle, dietary and clinical information in 216 hospitalized diabetic patients (171 men, 63 ± 9 years old and 45 women, 67 ± 5 years old) with a first event of an acute coronary syndrome (ACS) [myocardial infarction or unstable angina] and 196 frequency matched (by age and sex) diabetic controls (154 men, 64 ± 11 years old and 42 women, 66 ± 6 years old) without any clinical evidence of CHD (from the CARDIO2000 II study). Diabetes mellitus was defined according to the established ADA criteria. All participants were asked about their usual frequency of coffee consumption over the previous year (instant coffee, "Greek" type, "cappuccino", or filtered), adjusted to one cup of 150 ml coffee, with caffeine concentration of 27.5%. Conditional logistic regression analysis was used to evaluate the estimates of the relative risks of developing an ACS, by calculating odds ratios.
**Results:** We revealed a J-shaped association between the odds of having ACS and the daily quantity of filtered coffee consumption. In particular, the odds ratios for moderate (<300 ml/day), heavy (300–600 ml/day) and very heavy (>600 ml/day) consumption, relative to no consumption, were: 0.81 (p=0.023), 1.26 (p=0.081) and 3.10 (p=0.02), respectively, after controlling for the presence of hypertension, hypercholesterolemia, family history of premature coronary heart disease, physical activity status, smoking habits, body mass index, alcohol consumption, triglyceride levels, consumption of several food items, depression scale score and educational status. On the other hand, when we focused our interest on unfiltered coffee drinking, we observed a positive association with the occurrence of an ACS, without a J-shape. This kind of coffee drinking increased the odds of having an ACS by an average of 26% (odds ratio=1.26, p=0.04), with moderate, heavy and very heavy consumption increasing the odds by 7%, 12% and 34%, respectively.
**Conclusion:** Filtered coffee consumption shows a J-shaped association with the occurrence of an ACS in diabetic subjects. This may partially explain the conflicting results from other studies in the past, since unfiltered coffee drinking seems to be associated with adverse events in diabetic subjects.
*Supported by: Hellenic Heart Foundation*

## 802

**Effect of chromium chloride GTF milk powder supplement on Type 2 diabetic patients - a prospective, randomized, double-blind, placebo-controlled study**

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**Background and aims:** Type 2 DM is the fifth leading cause of death in Taiwan. The pathogens of DM are related to insulin resistance and abnormal insulin secretion. Most but not all diabetic patients' blood glucose can be effectively controlled with current available hypoglycemic agents. A biologically active chromium complex consists of one low-molecular-weight chromodulin and four chromiums. It activates insulin receptors through chromodulin to increase insulin signal transduction and insulin sensitivity. Animal studies showed that supplementing chromium could improve glucose and lipid metabolism. A human study by Anderson in 1997 showed that supplement of high dose of chromium could improve the control of DM.
**Materials and methods:** We (4 medical centers in Taiwan) conducted a randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of GTF-chromium milk powder in patients with type 2 DM. Total 120 type 2 DM patients, aged 30–75 years, under stable OHA dosage of gliclazide alone less than 160 mg/day for at least 3 months. Their HbA1 c: 7.5~12%, FPG: 140~250 mg/dl, BMI: 20~35 kg/m$^2$, TG≤400 mg/dl.Two treatment groups to receive either GTF-milk powder (chromium 200μg/20 gm milk powder) or placebo for 16 weeks. FPG and related biochemical data were assessed periodically. Safety was evaluated by the frequency of AE and abnormal laboratory results.
**Results:** The results showed there was a trend for GTF-group to have a lower FPG (–5.91 vs.–8.46 mg/dl, $p$=0.047) at the end of the study. But there were no significant changes in other metabolic parameters (HbA1 c, fructosamine, HOMA, beta-cell function). No obvious changes in serum lipid profiles (TC, TG), but both groups had increased HDL-C and decreased LDL-C of the study (NS). No obvious adverse events were observed in both groups, except mild complaints were noted in GTF-group: constipation (2.6%), diarrhea (1.3%), and dry mouth (1.3%).
**Conclusion:** In summary, there was a trend for type 2 diabetes patients to have lower FPG after receiving 16-weeks GTF-chromium milk powder supplement; it was safe and well tolerated.

## 803

**The effect of cannabis sativa on fat and glucose metabolism in obese and lean Wistar rats**

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**Background and aims:** *Cannabis sativa* has been used in indigenous medicine as a treatment for diabetes. The prevalence of obesity and Type II diabetes in South Africa has escalated in the black population over the past 20 years. This study investigated the effects of *Cannabis sativa* on lean and obese Wistar rats, to determine its effects on glucose and fat metabolism.
**Materials and methods:** Rats were randomly assigned to the lean and obese groups (minimum of 10 rats per group), the lean rats were maintained on standard rat chow, while obese rats were maintained on a Cafeteria diet over a 6 week period, prior to Cannabis treatment. A chloroform extract was completed on dried plant material, the resin formed after exposure to nitrogen gas, was resuspended in 1% Tween 80 in saline. Obese and lean experimental animals were treated every alternate day, over 28 days, with an equivalent of 5 mg/kg body weight THC via subcutaneous injection, for the first 5 injections, with the dosage being reduced to 2.5 mg/kg body weight THC thereafter. Control rats received the vehicle (1% Tween 80 in saline). An Accutrend Glucometer GC was used to determine blood glucose levels. Standard enzymatic microtiter plate assays were used to determine total cholesterol and triglyceride of individual plasma samples (Roche, Mannheim, Germany). Radioimmunoassays (Linco, St. Charles, Michigan, USA) were used to determine plasma insulin and leptin levels. All data are expressed as the mean ± SEM and statistical analysis was performed using the paired Student t-test, P< 0.05.
**Results:** The difference in body weight between pre- and post-experimental lean (49.23 ± 7.93g; P<0.01) and obese (61.2 ± 4.72g; P<0.004) control rats increased, while the experimental lean (23.39 ± 8.63g) and obese (15.93 ± 3.75g) groups showed no significant weight gain. These findings were supported by a reduction in epididymal fat deposits in experimental lean (5.58 ± 0.24g; P<0.05), and obese (8.86 ± 0.49g; P<0.04) groups relative to lean (6.72 ± 0.45g) and obese (10.78 ± 0.65g) controls. The initial reduction and subsequent gain in body weight in lean and obese experimental groups show a dose-dependent response to cannabis treatment. Total plasma cholesterol and triglyceride levels (P<0.05) decreased , except in obese experimental rats where triglycerides increased (P<0.02). There were significant reductions in plasma insulin levels in all groups, but no significant differences were found between post-experimental control and experimental groups. The same trend was reflected in plasma leptin levels that showed significant reductions within the obese control (P<0.01) and experimental (P<0.03) groups. However, these changes were not significant between the post-experimental obese control and experimental groups. Fasting blood glucose in the obese groups decreased (P<0.002), while the lean groups showed an increase, though not significant. Pancreata weights (1.62 ± 0.09g vs 1.94 ± 0.1g; P<0.05) and thigh muscle weights (4.72 ± 0.14g vs 4.22 ± 0.12g; P<0.05) differed in both obese groups.
**Conclusion:** The changes in adipose deposits in the experimental groups are characteristic to those reported in literature. Since insulin plays a significant role in glucose metabolism, the observed changes in fasting blood glucose and insulin levels, warrants further investigation.
*We acknowledge the University of Port Elizabeth and the National Research Foundation for their financial assistance.*

## 804

**Evaluation of a traditional medicine for diabetes *in vivo* and *in vitro***

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**Background and aims**: Traditional medicines (TM) represent a potential source of new leads for diabetes therapeutic agents. However, only a small number of TM have been comprehensively studied, and very little is known about their active compounds, mechanisms, and effective dose. We investigated a TM used to treat diabetes in a South Pacific community to evaluate its effects in both *in vivo* and *in vitro* models of diabetes, and to further chemically analyse its nature and identify active components.
**Materials and methods:** *In vivo*: The traditionally prepared TM was mixed with the normal chow diet of the animals at a dose of (1% w/w) and fed to *Psammomys obesus*, a polygenic model of obesity and type 2 diabetes, for an 11 day period. Body weight, food intake, and blood glucose and insulin concentrations were measured at the start and the end of the study.
*In vitro*: The TM was tested in cultured 3T3-L1 adipocytes for the effects on glucose uptake and incorporation of glucose into lipid. The TM was fractionated by reflux solvent extraction based on polarity and ion-exchange chromatography, and all fractions were tested for activity in the above bioassays. Data was analysed by ANOVA to test for effects of the TM and its extracts.
**Results:** When consumed with the diet at levels similar to those used by traditional practitioners, the TM significantly reduced blood glucose concentrations in both lean, non-diabetic and obese, diabetic *P. obesus* (by 22% and 31% respectively, $p<0.05$), without significant change in food intake or insulin levels compared to control animals. In 3T3-L1 adipocytes, the TM dose-dependently increased glucose uptake by up to 140% ($p<0.001$), and glucose incorporation into lipid by up to 50% ($p<0.009$) in the presence of 1 nM insulin. These results confirm the *in vivo* results and suggest that the TM in question contains an insulin-sensitising agent. Preliminary fractionation experiments showed that the active components of this TM are highly polar and carry a negative charge. Further studies to identify the chemical nature of the active components are ongoing.
**Conclusions:** The data from animal and cell culture models suggests that the TM under investigation possesses insulin sensitising activity. This suggests the potentiality of the TM as a novel treatment for type 2 diabetes.

## 805

**A novel insulin-releasing substance, phanoside, from the plant *Gynostemma pentaphyllum.***

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**Background and aims:** Extracts from *Gynostemma pentaphyllum* Makino (Cucurbitaceae), an East-Asian herb, have been reported to have numerous activities, such as antitumor, cholesterol-lowering, immunopotentiating, antioxidant and hypoglycemic effect. The aim of the present study was to purify and characterize the hypoglycemic compound in ethanol extract of *Gynostemma pentaphyllum*.
**Materials and methods:** We have isolated the active hypoglycemic compound by ethanol extraction, distribution in butanol/water, solid phase extraction/separation and several rounds of RP-HPLC. The effect of the compound on insulin secretion was investigated in isolated rat pancreatic islets. Then the structure of the active substance was determined by mass spectromatry and NMR techniques.
**Results:** We have shown by NMR and mass spectrometry analyses a novel insulin releasing saponin, a gypenoside that we named phanoside, (21-, 23-epoxy-, 3β-, 20-, 21-trihydroxydammar-24-ene-3-O-{[α-rhamnopyranosyl (1→2)] - [β-D-glycopyranosyl (1→3)]-β-D-lyxopyranoside)) with a molecular mass of 914.5 Da. Phanoside is a dammarane-type saponin and 4 stereoisomers differing in configurations at positions 21 and 23 were identified, each of which were found to stimulate insulin release from isolated rat pancreatic islets. We also found that the stereoisomers are interconvertible. Dose-dependent insulin-releasing activities at 3.3 mM and 16.7 mM glucose levels were determined for the racemic mixture containing all 4 stereoisomers. Phanoside at 500 μM stimulated insulin release 10-fold at 3.3 mM glucose and potentiated the release almost 4-fold at 16.7 mM glucose. At these glucose levels 2 μM glibenclamide stimulated insulin release only 2-fold. Interestingly, β-cell sensitivity to phanoside was higher at 16.7 mM than at 3.3 mM glucose, since insulin responses were significantly increased by phanoside below 125 μM only at high glucose levels.
**Conclusion:** From the plant *G. pentaphyllum* we have isolated a novel, biologically active substance with the molecular mass 914.5 Da, a gypenoside named phanoside, which is a potent initiator and potentiator of insulin secretion from rat pancreatic islets.
*Supported by: SIDA/SAREC and Swedish Diabetes Association*

## 806

**Slow vs fast proteins in the stimulation of beta cell response and the activation of the entero-insular axis in Type 2 diabetes**

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**Background and aims:** In healthy individuals, whey protein ingestion results in greater post-prandial amino acid (AA) concentrations than casein ingestion. In NIDDM subjects, beta-cell response may be preserved to protein/AA administration while being impaired to glucose. We tested whether whey protein ingestion can induce greater post-prandial AA levels and a better beta cell response than casein ingestion in NIDDM.
**Materials and methods:** A mixed meal (6 kCal/Kg Body Weight) containing ≈50% of kCal as protein (either whey protein, casein or a free AA mixture matching the AA composition of casein), was randomly administered on three different occasions to twelve NIDDM patients.
**Results:** Following whey protein, concentrations of total branched chain and essential amino acids were 25%–50% greater than after casein ($p<0.0001$), and similar to those observed after free AA. C-peptide, insulin and pro-insulin concentrations were greater (by 12–40%, $p<0.05$ or less) with whey than with casein, and similar with free AA. Glucagon and GIP responses did not account for the observed differences. Post-prandial glucose concentrations were similar with whey protein and casein, but lower with the free AA meal.
**Conclusion:** In conclusion, in NIDDM fast-absorbable dietary proteins result in greater postprandial aminoacidemia and a better beta cell secretion. Whether such an improvement can be maintained also chronically, possibly resulting in a better glycemic control, remains to be established.
*Supported by: NESTEC*

# PS 72

# Beta cell preservation

## 807

**Proinsulin to insulin molar ratio indicates the accelerated dysfunction of pancreatic beta cells in patients with Type 2 diabetes mellitus when treated with sulfonylureas**

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**Background and aims:** We investigated proinsulin and insulin secretions in patients with type 2 diabetes mellitus after long-term treatment with various oral hypoglycemic agents (OHAs).
**Materials and methods:** Intact proinsulin (IPI) and total proinsulin (TPI) were measured by CLIA method (MLT Co. Ltd.). In addition to 32 control subjects (N group), 164 type 2 diabetic patients were used and divided into 4 groups; 82 treated only with diet (D group), 24 treated with relatively large doses of sulfonylureas (SUs) (glibenclamide>=3.75 mg, gliclazide>=120 mg, glimepiride>=3 mg) (S group), 46 treated with low doses of SUs (M group) and 12 treated with other non-SUs (O group).
**Results:** There were no significant differences in BMI and the duration of the disease among diabetic groups. IPI was 5.0±3.5, 7.3±5.3, 12.3±8.7, 12.3±7.7 and 10.1±6.6 pmol/l in N, D, O, M and S groups, respectively. In all the groups treated with OHAs, IPI was significantly higher than that in N group ($P<0.01$, respectively) and there was a significant difference between D and M group ($P<0.0001$). On the contrary, IRI tended to decrease in N, D, O, M and S groups in this order (6.7±5.1, 6.2±6.4, 8.1±5.8, 6.5±5.0 and 4.3±2.9 μU/ml). Accordingly, TPI/IRI molar ratios were 0.27±0.15, 0.45±0.25 , 0.61±0.53, 0.68±0.36 and 0.79±0.48 in N, D, O, M and S groups, respectively, and thus significantly higher in all the diabetic groups than that in N group. There was no significant difference between D and O groups. IPI/IRI ratios are 0.15±0.08, 0.25±0.15, 0.29±0.2, 0.41±0.25 and 0.50±0.37 in N, D, O, M, and S groups, respectively, showing the significant elevation in all the diabetic groups, especially in M and S groups. There was also no significant difference in IPI/IRI ratios between D and O groups. In SU groups, when glycemic control was good ($HbA_{1C}<7.0\%$), TPI and IPI were significantly lower than those in the poorly controlled groups, and hence, TPI/IRI and IPI/IRI ratios tended to be lower.
**Conclusion:** The relative increase in proinsulin to insulin molar ratio was recognized as the doses of SUs increased, despite the relative decreases in insulin secretion. The results indicate that the exhaustion of beta cells could be accelerated after the long-term intensive treatment with SUs, and increased IPI/IRI ratio seems to indicate the beta-cell dysfunction rather than insulin resistance in type 2 diabetes mellitus.

## 808

**The improvement of first-phase insulin response is related to long-term glycemic control in newly diagnosed Type 2 diabetic patients by continuous subcutaneous insulin infusion(CSII)**

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**Background and aims:** In newly diagnosed type 2 diabetic patients with severe hyperglycemia, 2 weeks CSII can induce adequate glycemic control with improvement of β-cell function. But it's unclear whether this improvement can lead to long-term optimal glycemic control without medication. We designed this prospective study to investigate whether long-term optimal glycemic control could be achieved without medication in newly diagnosed type 2 diabetic patients treated with transient CSII and its possible mechanisms.
**Materials and methods:** 68 newly diagnosed type 2 diabetic patients (from 24 to 73 years) with severe hyperglycemia (fasting blood glucose, FBG≥11.1 mmol/L) have been treated with 2 weeks CSII for optimal glycemic control. After 2 weeks CSII, they were treated with diet and exercises and followed longitudinally. Intravenous glucose tolerance tests (IVGTT) were performed and FBG, postprandial blood glucose (PBG), glycosylated hemoglobin $A_{1C}$($GHbA_{1C}$), insulin and C-peptide were measured before and after CSII.
**Results:** All of the patients have been followed up longer than 12 months. Nearly half of the patients (32/68) have maintained optimal glycemic control without medication longer than 12 months (remission group), eight of them even longer than 20 months. Others (36/68) failed to maintain euglycemia in 12 months (non-remission group). There were no differences in age, body mass index (BMI), $GHbA_1C$ and FBG before CSII between two groups. After CSII, HOMA IS (FBG × fasting insulin/22.5) of the remission group was higher than that of the non-remission group (145.4±89.5 versus 78.4±68.5), so as the area under the curve of C-peptide and insulin during IVGTT (9.9±4.2 pmol/l/min versus 7.8±3.4 pmol/l/min, 197.2±72.5 pmol/l/min versus 160.7±66.1 pmol/l/min, respectively). Although the first-phase insulin response of the remission group was lower than that of the non-remission group (−316.1±214.9pmol/l/min versus −152.2±311.2pmol/l/min) before CSII, there was no difference of the first-phase insulin response between two groups after CSII. As a result, Δfirst-phase insulin response (the first-phase insulin response after CSII subtracted that before CSII) which meaned the restoration of the first-phase insulin response was markedly different (621.8±430.4pmol/l/min versus 387.3±428.8pmol/l/min).
**Conclusion:** Nearly half of the patients can maintain euglycemia longer than 12 months by transient CSII. The improvement of β-cell function, especially the restoration of the first-phase insulin response observed in the study is related to sustained euglycemia in the newly diagnosed type 2 diabetic patients.

## 809

**Reduction of exocrine pancreas function in Type 1 diabetes mellitus: correlation with residual beta-cell secretion and glycaemic control**

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**Background and aims:** Little attention is paid in everyday practice to exocrine pancreas function in the follow-up of Type 1 diabetes mellitus. In the present study, we aimed to evaluate in type 1 diabetic patients the relationships between residual beta-cell secretion, glycemic control and exocrine pancreas function measured by pancreatic elastase-1 (PE-1) in stools, a parameter showing good correlation with the gold standard of the secretin-cerulein test. We also evaluated the correlation between PE-1 and fecal fat excretion.
**Materials and methods:** The influence exerted by gender, body mass index (BMI), diabetes duration, daily insulin dose, C-Peptide and HbA1 c on PE-1 (ELISA, normal values >200 μg/g stools) was investigated in 67 type 1 diabetic patients, 35 men and 32 women, age 35±1.0 years, known diabetes duration 15.5±1.1 years, HbA1 c 8.5±0.19%, BMI 23.7±0.4, C-peptide 0.22±0.04 ng/ml. Twenty non diabetic subjects, 8 men and 12 women, age 33±3 years, BMI 22.9±1.5, C-peptide 2.28±0.25 ng/ml were used as controls. Relationships between PE-1 and fecal fat excretion were measured in 30 patients with known diabetes duration >15 years. Both controls and type 1 diabetic subjects were asymptomatic and tested negative for antibodies anti-transglutaminase, anti-gliadin and anti-endomysium, checked to exclude patients affected by celiac disease.
**Results:** PE-1 concentrations in stools were lower in diabetic patients than in controls (277±21 vs 438±38 μg/g stools; $p=0.0003$), and, in type 1 diabetic patients: i) were not influenced by BMI and daily insulin dose; ii) correlated with C-Peptide ($r=0.40$; $p=0.0008$): 251±21μg/g stools in patients with C-Peptide ≤0.5 ng/ml vs 424±56 μg/g stools in patients with C-Peptide >0.5 ng/ml; $p=0.0071$); iii) correlated with HbA1 c ($r=-0.37$, $p=0.0022$): 240±22 μg/g stools in patients with HbA1 c >8% vs 343±40 μg/g stools in patients with HbA1 c ≤8%, $p=0.0427$. In the multiple regression analysis with BMI, diabetes duration, C-Peptide and HbA1 c as independent variables, C-Peptide and HbA1 c remained significantly correlated with PE-1 (std coeff=0.36, $p=0.0047$ and −0.40, $p=0.0045$, respectively). A fecal fat excretion ≥6 g/day, indicating steatorrhea, was measured in 11 out of 30 patients with diabetes duration >15 years and in all those with PE-1<100 μg/g stools. PE-1 correlated with fecal fat excretion ($r=-0.37$, $p=0.0424$); patients with values ≥6 g/day had lower PE-1 than patients with normal fecal fat excretion (220.3±49.2 vs 336.8±31.7, $p=0.0477$).
**Conclusion:** This study shows that both residual insulin secretion and glycemic control play relevant and independent effects on exocrine pancreatic function in Type 1 diabetic patients, demonstrates the frequent occurrence of an asymptomatic exocrine pancreas deficiency in these patients, shows that about 30% of type 1 diabetic patients with a disease duration >15 years present steatorrhea: absence of steatorrhea, on the other hand, does not mean a normal exocrine pancreas function, but simply a >10% preservation of function. In conclusion, this study suggests that PE-1 detec-

tion in stools, a simple test for exocrine pancreatic function, should be introduced in the clinical follow-up of type 1 diabetic patients with severe beta-cell failure, to timely detect and treat pancreatic insufficiency.

## 810

**Long-term effect of rosiglitazone on pancreatic β-cell function and insulin resistance in older Type 2 diabetes mellitus patients treated with sulphonylurea**

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**Background and aims:** The long-term effect of rosiglitazone (RSG) combined with sulphonylurea (SU) on β-cell function was assessed in a 2-year double-blind study in older type 2 diabetes mellitus (T2DM) subjects.
**Materials and methods:** 215 diabetic subjects aged 59–89, male:female ratio 73:27, BMI 30.4 ± 4.7 kg/m$^2$ were randomized into either glipizide (GLIP) + placebo (n = 110) or GLIP + RSG (n = 115) with an uptitration regimen for study medication (if fasting plasma glucose [FPG] ≥ 10 mmol/l). Homeostasis model assessment (HOMA) β-cell function (HOMA-β), insulinogenic index (ISI), HOMA insulin resistance (HOMA-IR) and $HbA_{1c}$ were assessed at baseline (BL) and study end (End). ISI was normalized by HOMA-IR to allow for the reciprocal relationship between β-cell function and insulin sensitivity.
**Results:** After two years, RSG-treated patients had significant improvements in both insulin resistance and β-cell function by both HOMA-β and ISI/HOMA-IR (see Table).

**Baseline data and changes from baseline at 2 years**[1]

| Parameters | GLIP + Placebo | | Total RSG + GLIP | |
|---|---|---|---|---|
| | BL/End | % Change | BL/End | % Change |
| $HbA_{1c}$ (%) (mean ± SE) | 7.6/7.8 ± 0.1 | 0.13 ± 0.10 | 7.6/7.0 ± 0.1 | −0.65 ± 0.08* |
| HOMA-IR** | 5.1/6.0 ± 0.3 | 17.75 ± 6.33 | 5.09/4.4 ± 0.2 | −13.79 ± 3.82%* |
| HOMA-β ** | 53.4/56.9 ± 4.3 | 6.44 ± 7.97 | 53.4/83.2 ± 5.0 | 55.79 ± 9.44%* |
| ISI/HOMA-IR[2] ** | 3.4/2.9 ± 0.4 | −14.08 ± 11.65 | 3.4/3.8 ± 0.4 | 10.97 ± 12.01%* |

1. Results were derived from multivariate linear model analysis
2. Insulinogenic index: Δ Insulin (0–30 min)/Δ Glucose (0–30 min) from oral glucose tolerance test
* Significant comparison ($P < 0.05$) between treatment group and baseline
** Percent change based on geometric mean

**Conclusions:** RSG and SU combination therapy had a durable effect on reducing insulin resistance and improving β-cell function compared to SU alone. Thus, this combination may be a useful alternative for the treatment of older subjects with type 2 diabetes as it provides improved glucose control at the same time as reducing the loss of β-cell function that characterises the disease.

## 811

**A randomised, double blind, placebo-controlled trial on the effect of Fusidin in patients with newly diagnosed Type 1 diabetes: the FUSIDM trial**

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**Background and aims:** Several attempts have been made to preserve β-cells from destruction in newly diagnosed type 1 Diabetes (T1D). The immunomodulatory properties of Fusidin (FUS), a cytokine-modulating anti-staphylococcal drug, have been tested in different immunoinflammatory conditions, including T1D. Our study aimed to investigate the effect of FUS in the metabolic control and β-cell function in patients with newly diagnosed T1D receiving intensive insulin therapy.
**Materials and methods:** 28 newly diagnosed T1D patients were included (age ≥ 18 years; mean±SD: 27 ± 5, 11 women, two-tailed α: 0.05, 1-β: 80 %). After correction of initial metabolic disturbances, subjects were randomly assigned to two different treatment groups of 24 week's duration: Control (n=13), intensive insulin therapy plus placebo and FUSG (n=15) intensive insulin therapy plus 500 mg FUS t.i.d. GAD, IA-2 and IAA antibodies were measured. C-peptide was measured basally and after 6 min. of 1 mg i.v. glucagon at the beginning of the study and after 24/48 weeks. $HbA_{1c}$ was determined initially and after 12, 24, 36 and 48 weeks.
**Results:** There were not initial differences in clinical characteristics, clinical onset, $HbA_{1c}$ and β-cell function. Two subjects in group C and 3 in FUSG were excluded because lack of compliance with the protocol. $HbA_{1c}$ remained without differences between groups during the study (24 weeks, C: 5.6 ± 0.8 and FUSG: 5.3 ± 0.6 and 48 weeks, C: 5.7 ± 0.9 and FUSG: 5.5 ± 0.4 %). We did not observe differences in delta C-peptide (stim - basal) after 24 (C, 0.57 ± 0.40 and FUSG, 0.53 ± 0.42 ng/ml) and 48 weeks of follow-up (C, 0.45 ± 0.30 and FUSG, 0.35 ± 0.20 ng/ml). Despite this, at the end of the study β-cell function remained preserved in both groups.
**Conclusion:** The addition of 6 months of Fusidin therapy does not improve β-cell function over that seen with intensive insulin therapy alone in newly diagnosed T1D.
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## 812

**Factors influencing C-peptide level in the first year of Type 1 diabetes in children**

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**Background and aims:** Clinical manifestation of type 1 diabetes (T1D) in children is caused by an autoimmune process that for many years destroys the pancreatic β-cells and leads to gradual decrease in the insulin secretion. C-peptide level is the most reliable measurement for evaluation the endogenous insulin secretion in patients with type 1 diabetes (T1D). However, its level varies at the onset of this disease and during the first year of T1D.
The aim of this study was to evaluate putative factors, which could be applied to predict the C-peptide levels at the onset of T1D in children and after the first year of the disease.
**Materials and methods:** For this purpose, 201 type 1 diabetic children, mean age=9.6 ± 4.0 years, 78 female and 123 male were studied. The control group for genetic studies involved 109 healthy children, 41 female and 68 male, mean age=10.4 ± 3.4 years.
Fasting C-peptide level was determined by radioimmunoassay. Among the potential C-peptide influencing factors were considered: age at the onset, gender, polymorphism in genes established as predisposing to type 1 diabetes and/or involved in metabolism of adipose tissue, insulin requirement, HbA1 c, ketoacidosis and BMI and adiponectin.
**Results:** Logistic regression analysis showed that risk factors for prediction of C-peptide level below the normal range at the disease onset were as follows: age at the onset OR(95%CI)=75.5 (13.4–425.4); ketoacidosis OR(95%CI)=0.3 (0.1–0.6); HbA1 c level OR(95%CI)=0.03 (0.002–0.6) and adiponectin level OR(95%CI)=15.1 (0.92–246.6) (for global model $p<10^{-5}$). However, after 12 months of diabetes duration risk factors for C-peptide level were slightly different: age at the onset OR(95%CI)=91,0 (4,5–1817,7); insulin requirement OR(95%CI)=0.007 (7.9 × $10^{-5}$–0.1), HbA1 c level OR(95%CI)=0.001 (7 × $10^{-5}$–0.3) and *PPARG2* gene polymorphism OR(95%CI)=7.0 (1.5–33.7) (for global model $p<10^{-5}$).
**Conclusion:** In conclusion, the different factors may affect residual insulin secretion at the clinical onset of type 1 diabetes in children and after the first year of the disease.
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## 813

**Low fecal elastase-1 values do not reliably indicate exocrine pancreatic insufficiency in Type 1 diabetes mellitus**

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**Background and aims:** Using fecal elastase-1 (FE-1) estimation up to 40 % of type 1 diabetes mellitus patients have exocrine pancreatic insufficiency and expensive pancreatic enzyme substitution seems to be necessary (Z Gastroenterol 39:823,2001; Pancreatology 3:395,2003). This study aim was to compare the results of FE-1 estimation in diabetic patients (type 1) with

the secretin caerulein test (SCT), the "gold standard" for measuring exocrine pancreatic function.
**Patients and methods:** A SCT and two FE-1 estimations (Schebo-Tech, D-Wettenberg; Bioserv, D-Rostock; abnormal <200 µg pancreatic elastase-1/g stool) were performed in 33 consecutive patients with type 1 diabetes.
**Results:** The SCT was abnormal in 11 (33.3%) patients, fecal elastase-1 estimation was abnormal in 16 (48.4%; ScheboTech) and 9 (27,3%; Bioserv) patients, respectively. Fecal elastase-1 estimations (ScheboTech; Bioserv) were falsely abnormal in 41% and 23% of patients with normal SCT and falsely normal in 36% and 64% of patients with exocrine pancreatic insufficiency, respectively.
When the test results were evaluated in comparison (ScheboTech; Bioserv) the following values were reached: Sensitivity 64%; 36%; specificity 59%; 77%; positive predictive value 44%; 44%; negative predictive value 77%; 71%.
**Conclusion:** Fecal elastase-1 estimations are unreliable for detecting exocrine pancreatic insufficiency in type 1 diabetes mellitus and low values should not lead to pancreatic enzyme subscription.

## 814

**Classification of diabetes in young Asians based on islet autoimmunity and β-cell function**

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**Backgrounds and aims:** The Asian Young Diabetes (ASDIAB) project is a five-year prospective study on the clinical and immunological characterisation of diabetes in newly diagnosed young Asians. This paper aims at evaluating the aetiological classification of diabetes in these patients based on presence/absence of islet autoantibodies and beta cell function at disease presentation and one year.
**Materials and methods:** A total of 919 patients (from Beijing, Shanghai, Hong Kong, India, Malaysia and Singapore) with age at diagnosis 12–40 years and diabetes duration <12 months were recruited between 1997 and 1999. Complete information on autoantibodies to glutamic acid decarboxylase (GAD) and IA-2 and fasting C-peptide at baseline and 1 year were available in 633 patients. Antibody positivity (Ab+) was defined by presence of GADab and/or IA-2 ab. Poor beta-cell function was defined with fasting C-peptide <0.3nM at one year. T1DM was identified in patients Ab+ at diagnosis (irregardless of β cell function status) and in those Ab- at diagnosis and 1-year, but demonstrated poor beta-cell function at 1-year. Patients who were Ab- at diagnosis and 1-year but had good beta cell function (fasting C-peptide ≥0.3nM) at 1-year were classified as having type 2 diabetes (T2DM).
**Results:** 139 patients (22%) were classified as having T1DM. Of these, 90 were Ab+ and 49 were Ab- and had poor beta cell function. The remainder 494 patients (78%) were classified as having T2DM. The ethnic distribution of T1DM patients (73% Chinese, 16% Indians and 11% Malays) was similar to the T2DM. Compared to T2DM, T1DM patients were significantly younger at diagnosis (mean age 28.0 vs 32.9 yrs), leaner (mean BMI 21.5 kg/m$^2$ vs 25.9 kg/m$^2$ at diagnosis, 22.0 kg/m$^2$ vs 26.1 kg/m$^2$ at 1 year), and had significantly higher $HbA_{1c}$ (11.8% vs 9.7% at diagnosis ; 8.9% vs 8.0% at 1 year) . Median fasting C-peptides were significantly lower in T1DM than T2DM patients (0.2 vs 0.7 nM at diagnosis; 0.2 vs 0.8 nM at 1 year). T2DM were more insulin resistant than T1DM patients as assessed by HOMA index (median 5.8 vs 4.4 at diagnosis, 4.9 vs 3.4 at 1 year).
**Conclusions:** In Asians with young onset diabetes, assessment at diagnosis and one year for islet autoantibodies (GADab and /or IA-2Aab), together with estimation of β-cell function with fasting serum C-peptide levels, were useful for classifying patients as having T1DM and T2DM .
*Grant from Novo Nordisk Asia Pacific, Singapore*

## 815

**Diabetes mellitus in chronic pancreatitis patients – the role of β-cell failure**

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**Background and aims:** Diabetes mellitus is a common complication of chronic pancreatitis (CP), however the mechanisms leading to its development have not been fully clarified. The aim of the study was to assess β-cell function in CP patients with newly diagnosed diabetes mellitus.
**Materials and methods:** Three groups of subjects were studied: Group 1 - CP patients with newly diagnosed symptom-free diabetes mellitus, before administration of any antidiabetic agents (n=10, mean age 44.0 ± 9.3 years); Group 2 - CP patients with normal glucose tolerance (n=10, mean age 48.0 ± 9.3 years); and Group 3 – healthy age-matched controls (n = 10). Clinical characteristics of chronic pancreatitis as well as plasma glucose, insulin, C-peptide and amylin concentrations were assessed during the oral glucose tolerance test (OGTT) performed according to the WHO protocol. Area under the curves of plasma insulin ($I_{AUC}$) and amylin ($A_{AUC}$) were calculated.
**Results:** Mean plasma insulin in OGTT was similar in Group 1 and Group 2, but significantly lower than in Group 3 ($p<0.001$): 0 min – 5.7 ± 2.3 and 6.0 ± 3.2 vs 19.0 ± 7.6; 60 min – 28.7 ± 11.7 and 26.6 ± 7.8 vs 136,4 ± 45,7; 120 min – 24.4 ± 9.7 and 17.7 ± 6.7 vs 41.9 ± 34.0 mIU/l, respectively. Mean $I_{AUC}$ in Group 3 was approximately fourfold greater than in Group 1 and 2: 166.9 ± 53.4 vs 43.8 ± 16.8 and 38.8 ± 11.3 mIU/l/h, respectively. Similar trend was observed in plasma C-peptide. Also, there were no significant differences in mean plasma amylin between Group 1 and Group 2 subjects: 0 min – 6.9 ± 3.9 and 4.9 ± 2.6; 60 min – 8.9 ± 4.8 and 8.3 ± 3.2; 120 min – 12.4 ± 17.8 and 8.6 ± 4.5 pM/l as was in $A_{AUC}$: 18.5 ± 14.5 and 25.8 ± 27.1 pM/l/h, respectively ($p>0.05$). Severity of CP assessed according to Cambridge classification was similar in Group 1 and Group 2: 3.3 ± 0.8 vs 3.1 ± 0.9. However, CP patients with diabetes (Group 1) were treated for CP almost three times longer than non-diabetes patients (Group 2): 9.4 ± 5.3 vs 3.5 ± 1.4 years ($p<0.05$).
**Conclusion:** β-cell function in chronic pancreatitis is severely impaired, regardless of presence of diabetes mellitus. Therefore, in addition to β-cell failure the pathogenesis of chronic pancreatitis-associated diabetes is likely to involve other mechanisms. Duration of chronic pancreatitis is a significant predictor of the development of diabetes mellitus.
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# Inflammation in Type 2 diabetes

## 816

**Effect of rosiglitazone on insulin sensitivity, B-cell sensitivity and serum-adiponectin in HIV-infected lipodystrophic patients on antiretroviral therapy**

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**Background and aims:** HIV-infected patients treated with highly active antiretroviral therapy (HAART) exhibit insulin resistance associated with lipodystrophy. In patients with type 2 diabetes, glitazones such as rosiglitazone improve insulin resistance partially via an increase in adiponectin, which is accompanied by the accumulation of subcutaneous fat. The aim of the present study was to evaluate the effect of rosiglitazone on insulin and B-cell sensitivity and on adiponectin in 40 non-diabetic, lipodystrophic HIV-infected patients on HAART.
**Materials and methods:** The patients were randomised to receive either 4 mg rosiglitazone (R, n=23) or placebo (P, n=17) for 24 weeks. Before and following treatment of blood was drawn for measurement of hormones including adiponectin (representative subset of 10 patients each group) and an OGTT (75 gms) was performed to calculate insulin sensitivity (OGIS) and B-cell sensitivity (insulinogenic index [(I30-I0)/(G30-G0)]) by a validated model.
**Results:** Body weight, fat mass, fat free mass, trunkal fat mass and waist/hip ratio remained stable in R and P. While insulin AUC (area under the curve) during OGTT did not change in R (p=0.5) and P (p=0.46), there was a marginal reduction in glucose AUC in R (27 ± 2 to 23 ± 0.5 g/dl 3 h, p=0.05), however not in P (p=0.52). OGIS was not altered in R (p=0.95) and P (p=0.6). The insulinogenic index was higher (p=0.03) following treatment in R (1.26 ± 0.18 vs. 0.91 ± 0.12) and did not change in P (p=0.76), indicating an increased sensitivity of the B-cell to release more insulin for the same increment in glucose.
**Conclusion:** In conclusion, treatment of lipodystrophic HIV-infected patients on HAART with rosiglitazone increases adiponectin levels without affecting insulin sensitivity and improves B-cell sensitivity. These findings are different from those reported in HIV-negative, type 2 diabetic subjects and might be related to the unaffected lipodystrophy in HIV-patients.

## 817

**Fatty acids differently regulate IL-6 promoter: palmitate and stearate but not oleate increase IL-6 promoter activity**

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**Background and aims:** Increased levels of IL-6 were shown to predict type 2 diabetes. But, so far it is unknown which factors trigger this inflammatory process. Since nutrients like fatty acids might be responsible for this inflammation we investigated the effects on IL-6 promoter activity of the saturated fatty acids palmitate and stearate and the monounsaturated fatty acid oleate.
**Materials and methods:** The human IL-6 promoter region (-550- +61 bp) was amplified by PCR and subcloned into the luciferase reporter vector pGL3 basic (Promega). HEK cells were transfected using Fugene (Roche) and were thereafter stimulated with palmitate, stearate or oleate 100 nM bound to bovine serum albumin for 24 hours. Analysis was performed relative to the co- transfected renilla construct (Promega) and relative to the unstimulated control. Student's T-Test for paired analysis was performed and mean and SEM are indicated.
**Results:** The sequence of the constructs was controlled by sequencing and the constructs did only differ at the known SNP at position -174 (G or C). The $A_nT_n$ side more upstream to the polymorphism investigated was $A_8T_{12}$. Stimulating the construct containing C at -174 with palmitate increased promoter activity relative to control to 1.66 ± 0.11 (p<0.001, n=11). Stearate increased promoter activity to 1.43 ± 0.11 (p=0.005, n=8). In contrast, stimulation with oleate did not affect the activity of the promoter construct (p=0.7, n=8). The effect of both palmitate and stearate was significantly different from oleate (p=0.002 and p=0.007). The stimulatory effects of both palmitate and stearate was not significantly influenced by the C-174G polymorphism (p=0.4 for palmitate, p=0.5 for stearate).
**Conclusion:** In contrast to the saturated fatty acids palmitate and stearate which both activate the IL-6 promoter constructs oleate did not affect IL-6 promoter activity. The effects of both palmitate and stearate were not affected by the C-174G polymorphism. These different effects of fatty acids on promoter activity suggest different effects of diets. Under the hypothesis of an activated inflammation increasing diabetes risk different fatty acid composition of the individual diet might therefore effect individual diabetes risk.
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## 818

**Effects of lifestyle intervention and insulin treatment on PAI-1, Hs-CRP and TNF-α levels in patients with Type 2 diabetes**

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**Background and aims:** Reduced fibrinolysis and increased pro-inflammatory activity play key roles in the pathology of cardiovascular disease and type 2 diabetes. Plasminogen activator inhibitor-1 (PAI-1) is the most important inhibitor of fibrinolysis. High sensitivity C-reactive protein (Hs-CRP) and tumor necrosis factor-α (TNF-α) are markers of systemic inflammation. We have previously shown that it is possible to obtain the same reduction in $HbA1_c$ with lifestyle intervention as with insulin treatment and while the insulin treated patients increased in body weight during the intervention year, the patients attending the lifestyle intervention program lost weight. Here we wanted to assess changes in concentrations of PAI-1, Hs-CRP and TNF-α in type 2 diabetic patients following the same lifestyle intervention program, insulin treatment or the two treatments combined.
**Materials and methods:** Thirty-one subjects with tablet-treated diabetes, median age 58 (47–70) years, HbA1c 9.1 (8.1–9.8) % and BMI 30.0 (27.6–32.7) kg/m$^2$, were randomised to the following treatments for one year: 1) lifestyle intervention (L), 2) insulin treatment (I) and 3) combined treatment (L+I). Blood samples were drawn at baseline and at the end of the intervention year and PAI-1, Hs-CRP and TNF-α were measured with commercial methods. Results are given as median ($25^{th}$–$75^{th}$ centiles) and non-parametric statistics are used.
**Results:** The lifestyle intervention group had a significant reduction in the concentrations of PAI-1 and Hs-CRP: -10.7 (-20.8 – -3.1) U/mL (p<0.01) and -2.2 (-7.7 – -0.5) mg/L (p=0.02) respectively. In the L+I group a significant reduction in PAI-1 was observed (-8.3 (-14.9 – -1.3) U/mL, p=0.02). The changes in PAI-1 concentrations obtained in the two lifestyle intervention groups were significantly different from that observed in group I. There was also significant differences in changes in the levels of Hs-CRP between group L and the two insulin treated groups. Although the lifestyle intervention group tended to have a reduction in TNF-α concentrations compared with the two insulin treated groups, the differences between groups in changes in TNF-α were not significant.
Baseline levels of body weight correlated with baseline concentrations of PAI-1 in the whole group (r=0.46, p=0.04). Changes in body weight observed correlated with changes in TNF-α (r=0.36, p=0.05) and Hs-CRP (r=0.63, p<0.01), but not with changes in PAI-1.
**Conclusion:** Lifestyle intervention can reduce the levels of PAI-1, Hs-CRP and possibly TNF-α when compared with insulin treatment. The changes observed in levels of TNF-α and Hs-CRP were closely correlated with changes in body weight.
*Supported by: Norwegian Foundation for Health and Rehabilitation*

## 819

**C-reactive protein and fibrinogen are elevated in insulin-using type 2 diabetic patients with metabolic syndrome**

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**Background and aims:** Individuals with the Metabolic Syndrome (MetS) are considered to be at increased risk for development of cardiovascular disease. We assessed the relationship between MetS and markers of cardiovascular risk and glycaemic control in an insulin-using type 2 diabetic population.

**Materials and methods:** We examined baseline data from a study comparing clinical efficacy and safety of rosiglitazone in combination with insulin to insulin alone. This study is being conducted in the USA. Randomized subjects (n = 643) had been on insulin monotherapy (≥ 30 units/day) for at least 8 weeks prior to randomization. MetS was defined according to the World Health Organization criteria, with BMI >30 kg/m$^2$ used to define obesity.
**Results:** As shown in the table below, subjects with MetS had significantly higher mean C-reactive protein (CRP) and fibrinogen (FBN) than did subjects who did not meet MetS criteria:

| | MetS+ (n = 360) | MetS- (n = 283) | *P* |
|---|---|---|---|
| $HbA_{1c}$ (%) | 9.08 ± 1.286 | 9.02 ± 1.301 | 0.5397 |
| FPG (mmol/L) | 10.12 ± 3.39 | 9.92 ± 4.08 | 0.5005 |
| CRP (mg/L) | 7.54 ± 9.012 | 4.43 ± 5.989 | < 0.0001 |
| FBN (µmol/L) | 11.65 ± 2.92 | 10.68 ± 3.07 | < 0.0001 |
| WBC (x 10$^9$/L) | 6.85 ± 1.758 | 6.58 ± 2.121 | 0.0703 |

FPG, fasting plasma glucose; WBC, white blood cell. Values shown are mean ± standard deviation

No differences were observed in $HbA_{1c}$ or fasting glucose between subjects with and without MetS, while the difference in baseline white blood cell count between those with and without MetS approached statistical significance. As expected given the lipid criteria for MetS, there was an association between LDL particle density and MetS: 79.9% of subjects with predominately small dense LDL met MetS criteria, compared to 42.3% of subjects with predominately buoyant LDL ($P < 0.0001$).
**Conclusion:** Subjects meeting criteria for MetS had elevated CRP and FBN relative to those who did not have MetS, although levels of glycaemic control between the two groups did not differ. The increased levels of CRP and FBN in subjects with MetS, as well as the association between MetS and LDL particle size, are consistent with the increased risk for CV events associated with this condition.

## 820

**Effects of blood glucose control in Type 2 diabetic patients on serum adiponectin concentration - Comparison with high sensitive CRP and Interleukin 6 -**

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**Background and aim:** Adiponectin was discovered through cDNA cloning techniques and there is growing evidence that hypoadiponectinemia is involved in the pathogenesis of aterosclerosis and insulin resistance, but the correlation between serum adiponectin concentration and blood glucose control has been unclear. The aim of present study is to clarify effects of blood glucose control in type 2 diabetic patients on serum adiponectin concentration.
**Materials and methods:** Fifteen type 2 diabetic patients participated in this study ( nine men, six women , mean age 58 years old; body mass index or BMI, 25.7plus/minus 0.9 kg/m$^2$). 8 patients started insulin therapy, metformin were added in 3 patients, 4 had no changes of drug therapy. 4 had simple, 2 had preproliferative retinopathy. In nephropathy, 6 had stage 1, 6 had stage 2, and 3 had stage 3A nephropathy. All subjects were admitted for 10 days to Asahi General Hospital. Serum adiponectin levels were measured by ELIZA which detect the monomer of adiponectin (Otsuka, Tokyo Japan). IL-6 was also measured by chemiluminescent enzyme immunoassay (CLEIA) (Human IL-6 CLEIA, Fujirebio, Tokyo, Japan).
**Results:** Serum adiponectin concentration on admission, had a significant negative correlation with BMI (R= -0.755, P<0.01). In patients with hypertension had a tendency to decrease, but there was not a significant difference( P=0.07). Fasting plasma glucose level decreased significantly (216.0 plus/minus 19.8 to 137.6 plus/minus 7.5 mg/dl), BMI, Ht did not change significantly after control of blood glucose. Serum adiponectin concentration significantly decreased (9.20 plus/minus 2.10 to 7.82 plus/minus 1.56 micro g/ml)(P=0.013). High sensitive CRP and IL-6 concentration did not change significantly (192.9 plus/minus 52.5 to 158.8 plus/minus 46.3 mg/dl, 5,7 plus/minus 2.7 to 3.4 plus/minus 0.9 pg/ml).

## 821

**Assessment of inflammatory markers in patients with diabetes mellitus and chronic hepatitis C virus infection**

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**Background and aims:** Previous studies have suggested that systemic inflammation is involved in the pathogenesis of diabetes mellitus and its chronic complications. The influence of chronic hepatitis C virus infection together with diabetes mellitus on vascular complications needs further investigation. To characterize the inflammatory response in patients with diabetes mellitus and chronic hepatitis C we measured serum concentration of C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), vascular endothelial growth factor (VEGF) and soluble intercellular adhesion molecule-1 (sICAM-1).
**Materials and methods:** Blood samples were obtained from 41 patients, aged 49.7 ± 13.7 years, duration of diabetes 6.7 ± 7.5 years, duration of evident HCV infection (anti-HCV+, HCV-RNA+, histopathological changes in liver tissue) 4.5 ± 3.4 years, HbA1 c 7.8 ± 2.1 %, C-peptide 2.5 ± 2.1 ng/ml, serum alanine aminotransferase (ALT) 100.9 ± 9.5 IU/l. Results were compared with the control groups - healthy subjects and patients with Type 1 diabetes mellitus. High-sensitive CRP was measured by immunoradiometric assay, other markers by immunoenzymatic assay.
**Results:** We observed significant differences in inflammatory markers between study groups. In multiple regression test CRP concentration was correlated with the duration of diabetes ($p<0.05$) and hematocrit ($p<0.05$).

mean ± SD; Kruskal- Wallis Test; $p< 0.05$

| | Diabetes and HCV n=41 | Type 1 diabetes n=30 | Healthy subjects n=30 |
|---|---|---|---|
| hsCRP (g/l) | 1.51 ± 1.41* | 3.14 ± 2.36& | 1.20 ± 0.41 |
| TNF-α (pg/ml) | 3.72 ± 2.64# | 2.50 ± 0.70& | 1.57 ± 0.69 |
| sICAM-1 (ng/ml) | 477.25 ± 212.83*# | 265.59 ± 84.15 | 207.25 ± 58.39 |
| VEGF (pg/ml) | 340.41 ± 166.31# | 440.76 ± 218.72& | 179.55 ± 116.62 |

* diabetes and HCV vs Type 1 diabetes,
\# diabetes and HCV vs healthy subjects,
& Type 1 diabetes vs healthy subjects

**Conclusion:** The results show the significant role of hepatitis C virus infection in the development of systemic inflammation. It seems that hepatitis C impaired C-reactive protein synthesis and could weaken the role of CRP as a potential risk factor for coronary heart disease.

# PS 74

# CSII including children

## 822

**Improving glycaemic control in young adults with Type 1 diabetes**

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**Background and aims:** Young adults with Type 1 Diabetes often have poor glycaemic control and recent UK audits have demonstrated worrying amount of microvascular complications. Our aim was to undertake a retrospective audit of young people aged 16 to 25 years attending a university teaching hospital.
**Materials and methods:** Patients were identified and data obtained using our departmental electronic clinical database. Statistical significance was determined using unpaired and paired t tests.
**Results:** 226 patients were identified, 102 females and 124 males, with mean age 21.04 years and mean duration 9.03 years.
Mean HbA1 c (mHbA1 c) in the whole cohort was 9.43% (± 2.15%) with 8.4% having levels <7.0%. Sixteen patients (7.08%) had hypertension, and 17 (7.52%) had diabetic retinopathy.
131 patients attended clinic in 2003 and early 2004, and 78.6% had had a Diabetic Annual Review done. They had mHbA1 c of 9.12% (± 2.15%) while in the 95 who had not attended since 2002, mHbA1 c was 9.86% (± 2.07%) (p=0.01).
The mHbA1 c in patients receiving twice daily insulin (n=139) was 9.47% (± 2.27%) and on basal bolus insulin therapy (BBIT) (n=82) was 9.34% (± 1.96%). Five patients were on other insulin therapies. Complete pre and post BBIT data was available in 43 patients, the pre-therapy mHbA1 c being 9.66% (± 1.72%) and the post-therapy mHbA1 c being 9.00% (± 1.65%) (p<0.005).
16 young adults have attended a dose adjustment for normal eating (DAFNE) course. mHbA1 c pre and post DAFNE were 8.97% (± 1.71%) and 8.43% (± 1.01%) respectively (p=0.097). Of these, 13 had HbA1 c>7.5% pre DAFNE with mHbA1 c of 9.41% (± 1.59%), with their current mHbA1 c being 8.69% (± 1.33%) (p=0.055).
mHbA1 c levels in young adults by age showed a decreasing trend with increasing age. The levels were 10.50% (± 2.57%) in those aged 16 years, 9.45% (± 1.96%) in 18 year olds, 9.54% (± 1.92%) in 20 year olds, 9.04% (± 2.34%) in 22 year olds and 7.79% (± 1.63%) in the 24 year olds (n= 8, 26, 28, 27 and 10 respectively).
**Conclusion:** Although overall glycaemic control remains sub-optimal, there are grounds for optimism. There is better glycaemic control in young adults being actively seen in clinic, with improved control in patients on BBIT and a trend toward improved control following attendance at a DAFNE course. The majority are now receiving a Diabetic Annual Review and the microvascular complication rate is low. Moving out of adolescence itself is also associated with improved glycaemic control.

## 823

**Long-term treatment with insulin lispro reduces overweight problems in juvenile and adolescent diabetics**

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**Background and aims:** The prevalence of obesity is an inherent problem in juvenile and adolescent diabetics. One strategy to solve this problem could be treatment with insulin lispro, the first prandial short-acting insulin analogue allowing more flexibility in both eating habits and meal size.
**Materials and methods:** This open, controlled, prospective study compares the long-term effect of insulin lispro and human regular insulin on metabolic control, frequency of hypoglycaemia and body weight in an outpatient setting. Only patients on intensified therapy were included, receiving lispro before or after a meal or regular insulin before a meal and following snack. As basal insulin, patients injected insulin glargine once or NPH/Semilente twice daily.
**Results:** 62 patients aged >12 years (mean age 16.8 ± 3.5 years) comprised the lispro group and 60 patients (mean age 15.6 ± 3.0 years) the control group. Treatment with insulin lispro was for a mean of 3.7 ± 1.7 years vs. 3.6 ± 1.7 years in the matched control group. There were no significant differences in prandial or basal insulin dosages, frequency of symptomatic or severe hypoglycaemia or metabolic control. At the start of the study HbA1 c was 7.5 ± 1.2 % in the study group vs. 7.7 ± 1.2% in controls. HbA1 c increased during the study period to 7.7 ± 1.3 in lispro group and 8.2 ± 1.9% in control group (p=0.31). BMI values were adjusted for age and weight classes (under-, normal- or overweight). BMI in the study group increased from 23.3 ± 3.8 to 24.1 ± 3.7 kg/m$^2$ and in the control group from 22.3 ± 3.7 to 24.2 ± 3.9 kg/m$^2$. 25% of the control group changed from normal weight to overweight, but only 4.8% of the patients in the lispro group. The proportion of patients with normal weight during the study was approximately 60% and remained constant in the lispro group; however, the proportion of the overweight group increased from 25.0% to 43.3% in controls. The percentage change in BMI was significantly higher in the control group (median 7.6%) than in the lispro group (median 2.5%). Also the mean of the percentage change showed a significant difference between the two groups: 4.0 ± 10.6% in lispro patients and 9.4 ± 13.5 % in controls (p=0.0103). The Mantel Haenszel chi-squared test and Fisher's exact test (p= 0.0049/p= 0.006) revealed a significant difference between the groups.
**Conclusion:** Our data show that long-term treatment with insulin lispro is well accepted in juveniles and adolescents with IDDM displaying a tendency towards better metabolic control. In particular, fewer overweight problems and improved weight control can be achieved in patients of this age group on long-term treatment with the short-acting insulin analogue lispro.

## 824

**Efficacy and safety of insulin glargine in children and adolescents with Type 1 diabetes mellitus**

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**Background and aims:** Currently available long-action insulins do not provide a constant and reliable basal insulin level in patients with type 1 DM, especially in children and adolescents. The introduction of the first 24-hour duration basal insulin analog-insulin glargine open the new perspectives in the treatment of DM1.
**Objective:** To determine if the addition of insulin glargin (Lantus) could improve glicemic control in children and adolescents with type 1 DM.
**Materials and methods:** 6-months non-randomized study: 50 patients with DM type 1, (6–18 years, mean 12.8 ± 3.3, duration of diabetes -from 6 months to 14 years, mean 4.9 ± 3.9) treated with multiple daily insulin injections, in whom basal insulin was changed from NPH to glargine initiated as a single daily dose. The starting dose of insulin glargin at bedtime was 80% of the previous total dose of basal insulin. The doses of glargine and prandial insulins were adjusted according to fasting and postprandial glycemia, taking into account plasma glucose at the badtime.
**Results:** 49 patients completed the study (1 patients withdrew his consent). Mean HbA1 c levels at baseline, after 3 and 6 months were 9,1 ± 1,5%, 9,3 ± 1,8% and 8,4 ± 1,5% (p=0.009 vs baseline) respectively, fasting glycaemia – 10,8 ± 2,4; 8,6 ± 2,5 and 8,5 ± 2,5 mmol/L (p=0,05); glycaemia at 3 a.m. – 9,3 ± 2,9; 9,0 ± 3,2 and 8,6 ± 2,5 mmol/L (p=0,3). Dose of glargine insulin was 0.31 IU/kg at baseline and after 3 months and 0,32 IU/kg after 6 months. Episodes of severe hypoglycemia were not registered. The incidence of nocturnal hypoglycemia tended to decrease. Only one patient complained of burning at the injection site. Insulin antibodies titer decreased insignificantly after 6 months of treatment with insulin glargin.
**Conclusion:** Insulin glargine improves glycemia control in pediatric DM1 without increase in hypoglycemic episodes, insulins antibodies, insulin requirements and body mass index. Once-daily use of long-acting insulin analog caused reduction of HbA1 c level (after 6 months), fasting glycemia (after 3 months), provided safe blood glucose level at 3 a.m. and tendency to fewer episodes of nocturnal hypoglycemia.
*Supported by: Aventis Pharma*

## 825

**Pharmacokinetics and safety of insulin glulisine in children and adolescents with Type 1 diabetes**

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**Background and aims:** Children and adolescents have difficulty adjusting daily activities to fixed insulin dosing and dosing-meal intervals, and may, therefore, benefit particularly from the use of rapid-acting insulin analogues. The safety and pharmacokinetic (PK) properties of insulin glulisine (GLU) compared with regular human insulin (RHI) injected sc before a

meal in paediatric patients with Type 1 diabetes were, therefore, investigated.
**Materials and methods:** Ten children, male and female, aged 7–11 years (mean 10 years), and 10 adolescents aged 12–16 years (mean 15 years), were enrolled in a single-dose, double-blind, randomized, crossover study. Blood glucose (BG) levels of fasted patients were maintained at 5.6–8.9 mmol/L (101–160 mg/dL) with variable iv insulin infusions. GLU or RHI was injected sc (0.15 IU/kg) 20 minutes after cessation of insulin infusion; 2 minutes later a weight adjusted standardized liquid meal was served.
**Results:** Maximum serum concentration (INS-$C_{max}$) and area under the initial insulin concentration-time curve (INS-$AUC_{0-2\,h}$) were higher; mean residence time (MRT) was shorter; and baseline-corrected blood glucose excursions (BG-$AUC_{0-6\,h}$) were lower for GLU than for RHI. When analyzing children and adolescents separately, exposure to GLU was the same. The two age classes showed almost equal PK profile for GLU with a trend towards higher exposure in adolescents. In contrast, the comparison between age classes for RHI revealed ~60% higher exposure in adolescents. GLU was well-tolerated, and incurred less post-prandial glucose excursion compared with RHI.
**Conclusion:** The PK properties of GLU in paediatric patients are no different from those in adults without diabetes or adults with Type 1 diabetes, thus GLU classifies as a rapid-acting insulin analogue in paediatric patients.

| | Geometric mean | | |
|---|---|---|---|
| | GLU | RHI | Point estimate (95% CI) |
| INS-$C_{max}$ (μU/mL) | 58* | 33 | 171 (127, 229) |
| INS-$t_{max}$ (min)† | 54 | 66 | −8 (−24, 7) |
| MRT (min) | 88* | 137 | 64 (59, 70) |
| INS-$AUC_{0-2\,h}$ (μU.min/mL) | 5232* | 2994 | 169 (127, 224) |
| INS-$AUC_{0-6\,h}$ (μU.min/mL) | 8361 | 7052 | 116 (90, 150) |
| BG-$AUC_{0-6\,h}$ (mg.h/dL)‡ | 641* | 801 | 80 (67, 95) |

*p <0.05 (analysis of variance [ANOVA]); †median; ‡arithmetic mean; CI = confidence interval

*Supported by: Aventis Pharmaceuticals*

## 826

**Safety of insulin glulisine compared with insulin aspart administered by continuous subcutaneous insulin infusion (CSII)**
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**Background and aims:** This 12-week, European, multicentre, controlled, open-label, randomized (1:1), parallel-group trial was conducted to compare the safety of a new rapid-acting insulin analogue, insulin glulisine (GLU), with insulin aspart (ASP) used in CSII.
**Materials and methods:** Patients with Type 1 diabetes (n=59) and previous experience of CSII (mean $HbA_{1c}$ [GHb measured as $HbA_{1c}$ equivalents] 7.0%; mean age 45.8 ± 11.1 years; mean BMI 26.0 ± 4.2 kg/m$^2$) received GLU or ASP by CSII.
**Results:** Three pumps were used in the study: Minimed (n=18); Disetronic H-Tron (n=39); Disetronic D-Tron (n=2). There was a low rate of catheter occlusions in the GLU and ASP groups (0.08 ± 0.2 vs 0.15 ± 0.3 occlusions/month, respectively; mean difference −0.06; 95% CI: −0.19, 0.06). Catheter occlusions associated with unexplained hyperglycaemia occurred in 1 patient (ASP group). Unexplained hyperglycaemia in the presence or absence of pump occlusions occurred in 6 patients (20.7%) in the GLU group compared with 12 patients (40.0%) in the ASP group. Mean rate of catheter changes was similar in the GLU and ASP groups (14.1 vs 14.8 changes/month, respectively). Infusion site reactions were reported with similar frequency in the GLU and ASP groups (3 patients vs 4 patients, respectively). No cases of diabetic ketoacidosis were reported. There were no noteworthy between-treatment differences in terms of the frequency of hypoglycaemic episodes. Possibly related treatment emergent adverse events occurred in 3 patients (10.3%) for GLU versus 4 patients (13.3%) for ASP and there were no significant between-treatment differences in terms of $HbA_{1c}$ (endpoint 6.98% for GLU vs 7.18% for ASP; treatment difference −0.20; 95% CI: −0.55, 0.15). Mean daily insulin doses and seven-point blood glucose profiles were similar in both groups.
**Conclusion:** The results of this study support the safe use of GLU in CSII therapy administered via an external pump in patients with Type 1 diabetes.
*Supported by: Aventis Pharmaceuticals*

## 827

**What is the most effective method of determining an initial basal rate for CSII?**
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**Background and aims:** Establishing an initial basal rate is key to successful insulin pump treatment. Inappropriately calculated rates will have the negative physiological effects of causing hypoglycaemia or hyperglycaemia. This can be psychologically stressful for the patient embarking on the first phase of a major treatment change. A starting dose for basal rate initiation can be calculated utilising various methods, which incorporate calculations based on weight or total daily insulin dose (TDI) but often they provide unsatisfactory results that require substantial modification before use. This study was undertaken in order to assess how recommended methods for calculating a basal rate compare to the actual outcome in the clinical setting.
**Materials and methods:** Twenty-four patients with sub-optimal glycaemic control with a mean HbA1 c 7.8 (SD +/− 0.85, range 5.3–9.7) and BMI of 26.3 (SD +/− 5.7, range 19–41), 18 reporting frequent hypoglycaemic events, were converted from multi-daily insulin injections to CSII. The mean TDI prior to CSII was 70.5 units (SD +/− 48.9, range 27–262). Initiation rate aimed for a blood glucose range of 4–14 mmol/L within the first 24 hours. The rates were calculated based on cross-referencing weight calculations (0.22, 0.5 & 0.7 units/kg) compared with a rate based on a 30% reduction in TDI. As the clinical situation developed clinical judgment was applied. Each method was statistically compared to the actual rate, determining which could better deliver glycaemic stability.
**Results:** The basal rate that achieved target glycaemic control had a median value of 0.5 units per hour (SD +/− 0.18, range 0.2–0.9). Significant differences were found between this and in 3 of the theoretically based calculations ($p < 0.005$). However the method that had no significant difference (0.5 units per kg − 30%) had poor accuracy, as it achieved the same outcome as the set rate in only 8 attempts (33.3%). The correlation value was r = 0.76. The other methods correlations ranged from r = 0.62–0.63 and accuracy from 4.2–16.7%.
**Conclusion:** The 4 methods for calculating the basal rate were of no clinical value and in most instances provided a significant over estimation of the required basal rate of insulin. Selection of an appropriate basal rate can be influenced by the TDI pre CSII but may also be affected by other factors such as the type of diabetes, insulin sensitivity/resistance, the residual effect of longer acting insulins and life style events. Determination of an initial basal rate requires a conservative approach based on clinical experience.

## 828

**First experiences with the use of insulin aspart in diabetic patients treated with CSII regimen**
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A total of 59 diabetic patients on insulin pump regimens from 5 diabetology centres were monitored to assess the efficacy and safety of insulin aspart. 33 men and 26 women (57 type-1 diabetics and 2 type-2 diabetics) with a mean age of 41,2 ± 12,2 years were monitored for a 3 month period. The mean duration of diabetes was 18,8 ± 8,5 years. HbA1 c, fasting and postprandial glycaemia levels were measured upon initiation of the study and twice subsequently over the 3 month period. Furthermore, the occurrence of treatment complications were monitored throughout the entire duration of the study.
In patients treated with buffered human insulin (72,9% of patients) or insulin lispro (23,7%), the average daily dose prior the change of therapy was 38,4 ± 11,9 IU, (15,2 ± 5,5 IU in boluses and 23,2 ± 8,0 IU as basal dose). The average daily dose of insulin aspart after 12 weeks of monitoring was 36,9 ± 10,1 IU (14,8 ± 5,9 IU in boluses, 22,1 ± 6,1 IU as basal dose). Upon initiation of treatment with insulin aspart HbA1 c levels were 8,6 ± 1,4%. These levels decreased to 8,0 ± 1,1% after 3 months of insulin aspart treatment at $p<0{,}0001$. The fasting glycaemia decreased statistically insignificantly from 7,1 ± 3,0 mmol/l to 6,3 ± 2,2 mmol/l, postprandial glycaemia decreased statistically highly significantly ($p<0{,}0001$) from 9,7 ± 3,0 mmol / l l to 7,5 ± 2,1 mmol/l. Furthermore, the average BMI value decreased from 24,9 ± 3,3 kg/m$^2$ to 24,7 ± 3,3 kg/m$^2$ ($p<0{,}01$).
During the course of the study, the frequency of hypoglycaemias (≤ 3,5 mmol/l) detected at self-monitoring decreased from 8% to 2,9% from

the total number of measurements (p<0,01). During the course of the monitoring period, 1 ketoacidotic event was recorded in the entire monitored group; otherwise no serious complications in treatment with insulin pumps were recorded.
**Conclusion:** Insulin aspart was shown to be suitable in monitored patients treated with insulin pumps. After 3 months of insulin aspart administration in insulin pumps, patients experienced a significant improvement in HbA1 c values as a parameter of long-term control of diabetes. Furthermore postprandial glycaemias were decreased as well as the number of hypoglycaemic events. Finally, a slight decrease in BMI was observed.
During the course of the study, no increase in treatment complications related to insulin aspart was recorded. This study confirmed the results of previously published clinical trials.

## 829

**Baseline HbA1c determines efficacy of insulin pump therapy: a pooled analysis of studies of continuous subcutaneous insulin infusion vs multiple daily injection regimens using rapid-acting insulin analogues**
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**Background and aims:** Rapid-acting insulin analogues have emerged as the meal insulin of choice in both multiple daily injection (MDI) regimens and continuous subcutaneous insulin therapy (CSII) for type 1 diabetes.
**Materials and methods:** We performed a pooled analysis of randomized controlled trials that compared CSII and optimized MDI therapy using rapid-acting analogues in adults with type 1 diabetes.
**Results:** The 3 studies that met inclusion criteria provided data on 139 patients, representing 596 patient-months on CSII and 529 patient-months on MDI. Mean age was 38.5 with duration of diabetes of 18.0 years. The 3 studies differed significantly in mean baseline A1 c (7.95%, 8.20 % and 9.27%). The pooled estimate of treatment effect comparing the percentage reduction in HbA1 c by CSII with that by MDI (CSII - MDI) was 0.35% [95% CI (-0.10, 0.80)], using a random effect to account for heterogeneity between studies. When treatment effect was plotted against baseline A1 c, however, the relative benefit of CSII over MDI was found to increase with higher baseline HbA1 c. In the pooled analysis, the interaction between baseline HbA1 c and treatment modality emerged as an independent predictor of treatment effect (CSII - MDI) (p=0.002). A model derived from these data predicts that, in a patient with a baseline A1 c of 10%, CSII would reduce A1 c by 0.65 more than MDI. Conversely, there would be no A1 c benefit of CSII compared to MDI if baseline A1 c were 6.5%.
**Conclusion:** When using rapid-acting insulin analogues in CSII and MDI regimens in adult patients with type 1 diabetes, insulin pump therapy is associated with better glycemic control, particularly in those individuals with higher baseline HbA1 c. Thus, CSII is an important modality for implementing intensive therapy and may be uniquely advantageous in patients with poor glycemic control.

## 830

**Comparison of continuous subcutaneous insulin infusion (CSII) vs. multiple daily insulin injections (MDI) in regard to quality of life: results of the 5-Nations trial**
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**Background and aims:** Anecdotal information about the impact of CSII on quality of life suggests benefits over MDI in terms of lifestyle and treatment flexibility and general well-being. Recent studies have demonstrated improvements in coping ability and treatment satisfaction for adolescents opting to use pump therapy rather than multiple injections and increased patient preference for CSII in a randomized parallel study. However the impact of CSII on quality of life has not been assessed in a large randomized crossover trial. Therefore, the goal of the study was to determine whether CSII differs from MDI with respect to quality of life in people with type 1 diabetes.
**Materials and methods:** The 5-Nations trial was a randomized, controlled, crossover trial, running in 11 European centers. 272 patients have been treated with CSII or MDI during a 2-month run-in period followed by a 6-month treatment period, respectively. For the evaluation of the quality of life the diabetes quality of life questionnaire (DQoL), the SF-12 questionnaire, and an additional questionnaire assessing further aspects of quality of life related to lifestyle and therapy manageability have been used.
**Results:** The overall DQoL score was significantly higher for CSII at the end of treatment compared to MDI (75 vs. 71, p<0.001), indicating a positive impact on QoL. Whereas all scores deteriorated during MDI treatment, an improvement in all categories was observed with CSII. There were significant improvements in treatment satisfaction (p<0.001), treatment impact (p<0.001) and a significant reduction in diabetes-related worry (p<0.01) when using CSII compared to MDI. The SF-12 questionnaire showed no differences in perception of physical health, but a significant improvement in perception of mental health when using CSII compared to MDI (p<0.05). The analysis of the lifestyle and manageability questionnaire showed that patients perceived significantly more flexibility with regard to eating habits (p<0.001) and significant improvement in lifestyle flexibility and sleep patterns (p<0.001) when using CSII compared to MDI.
In respect to patients' therapy recommendation 92% of the patients recommended MDI at the start of the study. By the end of the study only 63% of those on MDI recommended MDI, compared to 100% of those on CSII recommending CSII (p<0.001).
**Conclusion:** CSII usage offers significant benefits over MDI for individuals with type 1 diabetes with improvement in significant parameters of patient's quality of life.
*Supported by: Disetronic Medical System*

## 831

**C-peptide and HbA1c levels at diagnosis indicate a different pathogenic process depending on the age at onset of Type 1 diabetes**
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**Background:** Retention of β-cell function in patients with type 1 diabetes is known to result in improved glycaemic control and reduce hypoglycaemia, retinopathy and nephropathy. HbA1 c is a highly valuable clinical measure of glycaemic control but it is an insensitive measure of β-cell function. Therefore knowledge of parameters that may influence retention of β-cell function is important.
**Aims of the study:** Investigate in a large cohort of consecutive newly diagnosed type 1 diabetic patients (n=450) the relationship between HbA1 c, baseline c-peptide secretion and the age of onset of the disease since this parameter may alter the rate of β-cell destruction.
**Patients and methods:** We studied 115 pre-pubertal patients with a mean age of 6.7 ± 1.2 yrs., age range 2–8 yrs. (Group A), 250 pubertal with a mean age of 11.9 ± 2.1 yrs., age range 9–16 yrs. (Group B) and 85 post-pubertal with a mean age of 20.5 ± 2.4 yrs., age range 17–24 yrs. (Group C).
**Results:** HbA1 c levels at diagnosis were not statistically different between groups (Group A=9.7%± 2 SD; Group B=9.9%± 2.5; Group C=9.2%± 2.4). C-peptide levels were significantly higher with an increasing age at diagnosis (Group A=0.17 nM± 0.1 SD; Group B=0.24 ± 0.2 SD; Group C=0.31 ± 0.2; p<0.001); moreover there was an inverse significant correlation between HbA1 c and c-peptide in pubertal (p=0.01) and post-pubertal (p=0.01) patients. Surprisingly, in pre-pubertal patients there was no significant correlation between HbA1 c and c-peptide at diagnosis.
**Conclusion:** These data suggest that in patients <9 yrs of age the lack of correlation between HbA1 c and c-peptide (with the lowest c-peptide at diagnosis) indicates that the process of β-cell damage is very destructive and unique in this age group, reflecting a different pathogenic disease process. Such finding must be taken into account when designing trials for protecting β-cell function in patients with recent onset of type 1 diabetes.

# PS 75

# Rapid-acting insulin analogues in Type 1 diabetes

## 832

**Pharmacokinetics of insulin glulisine in renally impaired patients without diabetes**

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**Background and aims:** The pharmacokinetics (PK) of insulin may be altered by renal impairment. This single-dose study assessed the PK of insulin glulisine (GLU) in subjects without diabetes with normal renal function (creatinine clearance [CC] >80 mL/min), moderate renal impairment (CC 30–50 mL/min) and severe renal impairment (CC <30 mL/min).
**Materials and methods:** Twenty-four subjects (eight per renal function class) with a mean age of 56 years and a mean BMI of 26 kg/m$^2$ were enrolled in this open-label, parallel group, two-way crossover, single-dose study. On treatment days, 0.15 IU/kg of GLU (a therapeutic dose used in patients with diabetes without renal complications) was administered subcutaneously 2 minutes before a standard meal. Blood samples were taken at specified times for evaluation of serum insulin levels. PK parameters assessed were: area under the serum insulin concentration-time curve (AUC) for 0–1.5 h, 0–2 h, 0–5 h and 0–end, maximum serum insulin concentration ($C_{max}$), and time to $C_{max}$ ($T_{max}$), with renal function (CC) as a continuous variable.
**Results:** There were no apparent differences in the GLU concentration-time profiles of the three renal function groups. Correspondingly, there were no correlations between CC and the parameters characterizing the rapid-acting properties of GLU ($AUC_{0-1\,h}$, $AUC_{0-1.5\,h}$, $AUC_{0-2\,h}$, $C_{max}$ and $T_{max}$). Predicted changes by the weak correlation between renal function and total GLU exposure ($AUC_{0-end}$) were within the conventional bounds of equivalence.
**Conclusion:** The PK of GLU are not altered by renal impairment and do not warrant dose adjustments beyond those conferred by underlying changes in insulin resistance that occur in patients with renal impairment.

| Parameter | Geometric mean (n=8 per renal function class) | | |
|---|---|---|---|
| | Normal function | Moderate impairment | Severe impairment |
| $AUC_{0-end}$ (μIU.min/mL) | 13215 | 18473 | 17650 |
| $AUC_{0-1.5\,h}$ (μIU.min/mL) | 6734 | 8470 | 6873 |
| $AUC_{0-2\,h}$ (μIU.min/mL) | 9005 | 11626 | 9622 |
| $AUC_{0-5\,h}$ (μIU.min/mL) | 13120 | 18412 | 16912 |
| $C_{max}$ (μIU/mL) | 108 | 131 | 108 |
| $T_{max}$ (min) | 56 | 58 | 68 |

*Supported by: Aventis Pharmaceuticals*

## 833

**Insulin dose and weight profiles of pre- and post-meal insulin glulisine in a basal-bolus regimen with insulin glargine in patients with Type 1 diabetes**

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**Background and aims:** People with diabetes may inject their prandial insulin post-meal rather than pre-meal, to adjust for individual needs. However, few studies have evaluated this practice. This open-label, multinational, three-arm, randomized, controlled, parallel-group, 12-week study compared the efficacy of a new rapid-acting, human insulin analogue, insulin glulisine (GLU), injected 0–15 minutes before meals or post-meal (either immediately upon meal completion or 20 minutes after starting meals), with regular human insulin (RHI) injected 30–45 minutes before a meal, plus once-daily insulin glargine (LANTUS®) injected at bedtime.
**Materials and methods:** Patients with Type 1 diabetes (n=860; mean age 40.3 ± 11.7 years; mean BMI 27.1 ± 4.7 kg/m$^2$; mean $HbA_{1c}$ 7.7 ± 0.91% for GLU, 7.6 ± 0.92% for RHI) were given sc injections of pre-meal GLU (n=286), post-meal GLU (n=296), or pre-meal RHI (n=278). Insulin doses were titrated to achieve blood glucose targets while avoiding hypoglycaemia.
**Results:** Baseline to endpoint changes in $HbA_{1c}$ were similar for post-meal GLU and pre-meal RHI (–0.11% vs –0.13%, respectively); the greatest reduction was seen for pre-meal GLU (–0.26%). Bolus insulin dose increased from baseline in the pre-meal RHI group by 1.75 IU, but decreased for both pre-meal GLU (–0.88 IU) and post-meal GLU (–0.47 IU) groups. These between-treatment differences were significant (p=0.0001 and p=0.0012, respectively). Baseline to endpoint change in total insulin dose was greater for pre-meal RHI (2.35 IU) versus pre-meal GLU (0.04 IU; p=0.0042) and post-meal GLU (–0.22 IU; p=0.0014). Insulin glargine dose increased by <1 IU during the study in all groups. Hypoglycaemia rates were similar; however, fewer GLU patients in either dosing regimen experienced severe hypoglycaemia versus pre-meal RHI (8.4%, 8.4% and 10.1%, respectively). Body weight increased +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).
**Conclusion:** Glycaemic control with pre- or post-meal GLU is as effective as RHI, with no increase in total insulin dose. In addition, post-meal GLU is not associated with weight gain.
*Supported by: Aventis Pharmaceuticals*

## 834

**Prandial blood glucose control with pre- and post-meal insulin glulisine versus regular human insulin**

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**Background and aims:** The timing of insulin injections in relation to meals can impact prandial blood glucose control. The aim of this single-dose, randomized, four-way complete crossover study was to compare post-prandial blood glucose after pre- and post-meal sc injections of a new insulin analogue insulin glulisine (GLU) and regular human insulin (RHI) in patients with Type 1 diabetes.
**Materials and methods:** Twenty subjects (mean age 36 years; mean BMI 26.0 kg/m$^2$) were included in the study. Prior to a meal challenge, blood glucose levels were adjusted to 6.67 mmol/L (120 mg/dL) by variable iv insulin infusion. Subjects received a standardized meal and 0.15 IU/kg injection of either GLU (immediately before or 15 minutes after meal) or RHI (30 minutes before or immediately before meal).
**Results:** The pharmacokinetics of GLU were independent of meal time, and equivalent whether injected pre- or post-meal. GLU injected immediately pre-meal afforded about equal blood glucose exposure (area under the blood glucose concentration-time curve at 0–6 h [BG-$AUC_{0-6\,h}$]; maximum blood glucose excursion [$\Delta BG_{max}$]) to RHI injected 30 minutes pre-meal. Given immediately pre- or post-meal, GLU better mimics physiological post-prandial glucose disposal with a shorter time to $\Delta BG_{max}$ ($t\Delta BG_{max}$) and less post-prandial sway than RHI.
**Conclusion:** Both pre- and post-meal administration of GLU is at least as effective at controlling prandial blood glucose as RHI in individuals with Type 1 diabetes.

| | GLU | RHI | | |
|---|---|---|---|---|
| | Pre | 15 min post | 30 min pre | Pre |
| INS-$C_{max}$ (μIU/mL) | 82 | 79 | 46 | 45 |
| INS-$AUC_{0-6\,h}$ (mIU.min/mL) | 11.9 | 11.9 | 11.6 | 11.5 |
| INS-$t_{max}$ (min)* | 55 | 57 | 82 | 97 |
| $\Delta BG_{max}$ (mg/dL) | 65 | 84 | 64 | 89 |
| $t\Delta BG_{max}$ (min)* | 48 | 45 | 115 | 70 |
| BG-$AUC_{0-6\,h}$ (mg.h/dL) | 708 | 777 | 715 | 770 |

All values are mean unless *median stated; INS = insulin; $\Delta BG_{max}$ is baseline corrected

*Supported by: Aventis Pharmaceuticals*

## 835

**Efficacy and safety of insulin glulisine and insulin lispro combined with insulin glargine in patients with Type 1 diabetes**

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**Background and aims:** This 26-week, multinational, multicentre, randomized, controlled, open, parallel-group study compared the efficacy and safety of insulin glulisine (GLU), a new rapid-acting human insulin analogue, with insulin lispro (IL) each in combination with insulin glargine (LANTUS®; GLAR).

**Materials and methods:** Patients with Type 1 diabetes received GLU (n=339) or IL (n=333) 0–15 minutes before meals, with once-daily GLAR, all titrated to prespecified blood glucose (BG) targets, avoiding hypoglycaemia. Efficacy measures were baseline to endpoint change in $HbA_{1c}$ (GHb measured as $HbA_{1c}$ equivalents), BG parameters, hypoglycaemia and insulin dose.

**Results:** Baseline data were similar in both groups. In the GLU group, however, there was a significantly, ~2 years, longer diabetes duration than in the IL group. GLU and IL reduced $HbA_{1c}$ to a similar extent (GLU: baseline 7.6 ± 0.05%, change −0.14 ± 0.04% vs IL: baseline 7.6 ± 0.05%, change −0.14 ± 0.04%, respectively). Doses of both rapid-acting insulins decreased during the study (−1.07 ± 0.42 IU for GLU vs −0.81 ± 0.43 IU for IL; p=NS); however, there were significant between-treatment differences in change in total daily insulin (mean change −0.86 ± 0.54 IU for GLU vs +1.01 ± 0.54 IU for IL; p=0.0123) and GLAR dose (mean change +0.12 ± 0.31 IU for GLU vs +1.82 ± 0.31 IU for IL; p=0.0001). Self-monitored BG values and 2-hour mealtime glucose excursions were similar in both groups. Rates of symptomatic hypoglycaemia were similar for GLU and IL (3.64 ± 4.49 vs 3.48 ± 4.38 episodes/patient month, respectively), as were rates of severe (0.03 ± 0.12 vs 0.02 ± 0.12 episodes/patient month), nocturnal (0.55 ± 0.94 vs 0.53 ± 0.84 episodes/patient month) and severe nocturnal hypoglycaemia (0.01 ± 0.05 vs 0.01 ± 0.05 episodes/patient month). No between-treatment differences were noted for injection site abnormalities (11 patients [3.2%] and 14 patients [4.2%] for GLU and IL, respectively). Median anti-insulin antibody formation was similar and decreased in both groups (baseline 0.7% bound/total [B/T]; change −0.02% B/T for GLU; baseline 0.89% B/T; change −0.175% B/T for IL). These similar safety profiles persisted in a 26-week extension of this study (total treatment period 52 weeks; n=589). Specifically, there were decreases in median cross-reactive insulin antibodies for GLU (baseline 0.7% B/T; change −0.21% B/T) and for IL (baseline 0.88% B/T; change −0.26% B/T) and no between-treatment differences in injection site abnormalities (19 patients [5.6%] and 22 patients [6.6%] for GLU and IL, respectively) were noted over 52 weeks.

**Conclusion:** GLU is as effective and well tolerated as IL, using less total daily insulin, in a basal-bolus regimen with GLAR in patients with Type 1 diabetes.

*Supported by: Aventis Pharmaceuticals*

## 836

**Efficacy and safety of post-meal insulin glulisine compared with pre-meal regular human insulin in a basal-bolus regimen with insulin glargine**

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**Background and aims:** The timing of insulin injections in relation to meals is an important consideration for patients with Type 1 diabetes. This open-label, multinational, three-arm, randomized, controlled, parallel-group, 12-week study compared the efficacy and safety of insulin glulisine (GLU), a new rapid-acting, human insulin analogue injected 0–15 minutes before meals and post-meal (either immediately upon meal completion or 20 minutes after starting the meal) with regular human insulin (RHI) injected 30–45 minutes before a meal.

**Materials and methods:** Patients with Type 1 diabetes (n=860) receiving once-daily insulin glargine (LANTUS®) at bedtime (mean age 40.3 ± 11.7 years; mean BMI 27.1 ± 4.7 kg/m²; mean $HbA_{1c}$ 7.7 ± 0.91% for GLU, 7.6 ± 0.92% for RHI) were given subcutaneous injections of pre-meal GLU (n=286), post-meal GLU (n=296), or pre-meal RHI (n=278). Insulin doses were titrated to achieve blood glucose targets (fasting blood glucose 5.0–6.7 mmol/L for insulin glargine and 2-hour post-prandial blood glucose 6.7–8.9 mmol/L for GLU and RHI), avoiding hypoglycaemia.

**Results:** The greatest baseline to endpoint $HbA_{1c}$ reduction was seen with pre-meal GLU (−0.26%); $HbA_{1c}$ improvements were similar for post-meal GLU (−0.11%) and RHI (−0.13%) (Table). Post-prandial blood glucose values with pre-meal GLU were significantly lower than in the other groups at 2-hours post-breakfast (p=0.0001 vs RHI; p=0.0017 vs post-meal GLU) and 2-hour post-dinner (p=0.0001 vs RHI; p=0.0137 vs post-meal GLU). Symptomatic hypoglycaemia rates were similar. The incidence of severe hypoglycaemia was similar for pre-meal and post-meal GLU, and slightly higher for RHI (8.4%, 8.4% and 10.1%, respectively).

**Conclusion:** GLU can be administered safely and effectively pre-meal or post-meal.

**Table. Change in $HbA_{1c}$ (%) from baseline to endpoint**

| Timepoint | Pre-meal GLU | Post-meal GLU | RHI | Between-treatment difference | | |
|---|---|---|---|---|---|---|
| | (n=268) | (n=276) | (n=257) | Adjusted mean | 98.33% CI | p value* |
| Baseline† | 7.73 | 7.70 | 7.64 | - | - | - |
| Endpoint† | 7.46 | 7.58 | 7.52 | - | - | - |
| Change† | −0.27 | −0.12 | −0.12 | - | - | - |
| Postmeal GLU vs RHI Adjusted mean change‡ | - | −0.11 | −0.13 | 0.02 | −0.11, 0.16 | 0.6698 |
| Premeal GLU vs RHI Adjusted mean change‡ | −0.26 | - | −0.13 | −0.13 | −0.26, 0.01 | 0.0234 |
| Postmeal vs premeal GLU Adjusted mean change‡ | −0.26 | −0.11 | - | 0.15 | 0.02, 0.29 | 0.0062 |

*Statistically significant if p <0.0167

†Mean values

‡p values and adjusted means from analysis of covariance model (analysis of variance for baseline)

CI = confidence interval

*Supported by :Aventis Pharmaceuticals*

## 837

**Comparison of a multiple daily injection regimen with once-daily insulin glargine basal insulin and mealtime lispro, to continuous subcutaneous insulin infusion: a randomized, open, parallel study**

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**Background and aims:** Meta-analysis suggests continuous subcutaneous insulin infusion (CSII) is superior to multiple daily insulin injections (MDI) using NPH as basal insulin. The aim of this multicentre study was to establish whether MDI using the long-acting analog insulin glargine once-daily (with meal-time lispro) achieves glycaemic control ($HbA_{1c}$) equivalent to CSII (lispro).

**Participants and methods:** People with Type 1 diabetes ($HbA_{1c} \leq 9.0\%$) naïve to CSII and glargine were randomized and treated for 6 months with CSII (N=28) or MDI (N=29).

**Results:** $HbA_{1c}$ decreased from 7.7 ± 0.7 (SD) to 7.0 ± 0.8 % with CSII, and from 7.8 ± 0.6 to 7.2 ± 0.7 % with MDI, the baseline/centre adjusted difference being −0.1 (95% CI −0.5, 0.3) % (CSII vs. MDI, NS). Mean daily blood glucose (BG) level decreased from 9.1 ± 2.2 to 8.1 ± 1.7 mmol/l and from 8.8 ± 1.6 to 8.0 ± 1.1 mmol/l respectively (difference 0.05 (−0.7, 0.8) mmol/l (NS)). The MAGE decreased from 8.0 ± 2.3 to 6.3 ± 2.2 mmol/l (CSII) and from 7.6 ± 1.7 to 6.3 ± 2.1 mmol/l (MDI) (CSII vs MDI, NS). Coefficient of variation of eight-point BG profiles decreased from 53 ± 10 to 46 ± 8 % and from 52 ± 12 to 47 ± 11 % (NS). Confirmed hypoglycaemic events per patient (BG <4.0 mmol/l) over the 6 months were not statistically different (41 ± 8 (SE) vs 35 ± 7 events, CSII vs MDI, NS). Severe hypoglycaemia was too infrequent to allow meaningful comparison (2 events in all). Average cost per treatment was ~4 times more expensive with CSII.

**Conclusions:** both CSII and a once-daily glargine-based MDI regimen improve BG to a similar extent with no differences in mean BG, $HbA_{1c}$, BG excursions, and frequency of hypoglycaemia. A glargine-based MDI regimen is less expensive and therefore more cost-effective when used in an unselected population of people with Type 1 diabetes.

## 838

**Modeling and simulation of post-prandial feed back control for different size meals in Type 1 diabetics**

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**Background and aim:** To explore by simulation the capability of a closed loop artificial pancreas to control postprandial glycaemia in type 1 diabetic patients. The effect of closed loop control was compared to the standard continuous subcutaneous insulin infusion (CSII) therapy in response to meals of different size.
**Materials and methods:** A dynamic Proportional and Derivative controller on the second derivative (P-D-$D^2$) was feed back connected to a previously developed model of diabetic patient. The simulated response to different size meals (45, 90 e 135 g of carbohydrates) of the control system was compared with the simulated response to standard (bolus plus basal insulin infusion) CSII therapy. The performance indexes chosen were the area under the curve of glycaemic profile above 100 mg/dl (QG) and the hyperglycaemia duration over 100 mg/dl (Durat). The hypoglicaemia threshold was set at 55 mg/dl.
**Results:** Simulation's results are reported in the following table, were Gmax and Gmin are respectively the maximum and the minimum of glycaemic profile.
**Conclusions:** In our simulation study the responses to light and normal meals ( 45 - 90 g of carbohydrates) show a satisfactory post-prandial glucose control, a reduced QG and a reduced Durat, when treated with the P-D-$D^2$ feed back controller in respect to CSII, without decreasing below the hypoglycaemia threshold.
However when the carbohydrates amount is further increased, the feed back control alone is not able to satisfactory manage the post-prandial hyperglycaemia without the risk of late hypos. The combination of the feed back control with a reduced pre-prandial bolus might be a strategy for improving post-prandial control realized by standard therapy. These results indicate that the limitations deriving from the glucose variations as the driving element for the feed back control, become evident in presence of high carbohydrates intake.

| CASE | Carbo-hydrates (g) | Bolus (U) | Total insulin infused (U) | Gmax (mg/dl) | Gmin (mg/dl) | QG (mg*h/dl) | Durat (h) |
|---|---|---|---|---|---|---|---|
| **90 g meal bolus** | 90 | 5.00 | 5.00 | 164 | 84 | 138 | 3.95 |
| **90 g meal feed back** | 90 | 0 | 7.32 | 168 | 70 | 100 | 2.54 |
| **45 g meal bolus** | 45 | 2.50 | 2.50 | 126 | 89 | 50 | 3.38 |
| **45 g meal feed back** | 45 | 0 | 2.96 | 128 | 89 | 37 | 2.22 |
| **135 g meal bolus** | 135 | 7.50 | 7.50 | 202 | 80 | 217 | 4.10 |
| **135 g meal feed back** | 135 | 0 | 12.40 | 208 | 55 | 159 | 2.64 |
| **Feed back + bolus** | 135 | 4.00 | 9.23 | 204 | 75 | 173 | 2.73 |

## 839

**Comparison of insulin glargine injection at lunchtime, dinner-time and bedtime in people with Type 1 diabetes using mealtime insulin lispro**

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**Background and aims:** In some people with Type 1 diabetes using insulin glargine, the profile of action may be insufficient to provide optimal blood glucose control to the end of each 24-h period after injection, or in the period 1–4 hours immediately after injection. In such people, injection of insulin glargine earlier than bedtime might improve 24-h blood glucose control.
**Materials and methods:** In this 16-week, single-centre, open, randomized, three-way cross-over study, people with Type 1 diabetes (n=22, baseline $HbA_{1c}$ 8.3 ± 0.9 (± SD) %) were randomized to injection of insulin glargine at lunchtime (mean 12:37 ± 00:34 (± SD) h), dinner-time (18:12 ± 00:40 h), or bedtime (22:29 ± 00:40 h). Mealtime insulin was insulin lispro throughout. Insulin doses were titrated to target self-monitored blood glucose (SMBG) concentrations that were identical for each 4-week treatment period. Each period concluded with a 24-h in-patient metabolic assessment. Treatment satisfaction was assessed at the end of each period with the Diabetes Treatment Satisfaction Questionnaire (DTSQ). Primary outcome was mean in-patient plasma glucose concentration at 2200–0200 h.
**Results:** Twenty people completed the study. $HbA_{1c}$ reduced to 6.9 ± 0.1, 7.0 ± 0.1 and 6.8 ± 0.1 (± SE) % with lunch, dinner and bedtime insulin glargine, without a difference between treatment periods. Insulin doses and fructosamine concentration did not differ between treatment periods. Pre-breakfast SMBG concentration was higher with lunch than dinner or bedtime glargine (9.2 ± 0.3 vs 8.2 ± 0.3 or 8.0 ± 0.3 mmol/l, p=0.016), as probably was pre-lunch SMBG (8.6 ± 0.7 vs 6.4 ± 0.7 or 6.4 ± 0.8 mmol/l, p=0.051). Pre-dinner SMBG level was higher with dinner than lunch or bedtime glargine (9.4 ± 0.9 vs 4.9 ± 0.9 or 7.4 ± 1.1 mmol/l, p=0.007). In-patient plasma glucose concentration at 21:15–23:45 and 02:15–04:15 h was higher with bedtime glargine compared to the other groups. For 2200–0200 h mean in-patient plasma glucose concentration was higher with bed than lunch or dinner-time glargine (9.1 ± 0.6 vs 7.8 ± 0.6 or 6.7 ± 0.6 mmol/l, p=0.023). Serum free insulin concentration was lower at the end of the afternoon with dinner than lunch or bedtime glargine (11.5 ± 1.4 vs 20.2 ± 1.3 or 16.5 ± 1.3 mU/l, p<0.001). Frequency of hypoglycaemia was not different but timing of hypoglycaemia differed between treatment periods with a distinct peak and trough time of hypoglycaemia being observed in each treatment period. Treatment satisfaction (DTSQ), and convenience of timing of insulin glargine injection did not differ between treatment periods.
**Conclusion:** Blood glucose levels rise around the time of injection of insulin glargine whether given at lunchtime, dinner-time or bedtime. This suggests that once daily insulin glargine does not provide completely optimal 24-h basal insulin supply when used with a mealtime rapid-acting insulin analogue in some people with Type 1 diabetes. Bed-time injection leads to hyperglycaemia in the early part of the night, which is improved by giving insulin glargine at lunch or dinner-time. This study provides evidence of a significant maximum in glucose-lowering activity with insulin glargine and waning before the next injection is due, suggesting that different individuals may benefit from different times of injection of insulin glargine according to individual hypoglycaemia profile.
*This study was independent and not supported by the pharmaceutical industry.*

## 840

**Short acting insulin analogues in adult patients with Type 1 diabetes: meta-analysis with respect to study publication date**

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**Objectives:** To compare data on the effect of treatment with short acting insulin analogues (SAI-analogues) versus regular insulin on glycaemic control and on the risk of hypoglycaemic episodes in Type 1 diabetic patients depending on publication date.
**Methods:** Assessing the effect of SAI-analogues versus regular insulin in adult patients with Type 1 diabetes mellitus with respect to publication date (published year <2000 or year ≥ 2000) of the article reporting the data. Literature search until December 2003 was performed for randomised controlled trials with an intervention duration of at least four weeks or more. Insulin had to be injected subcutaneously and any additional treatment given equally to both groups. The review was performed according to the guidelines of the Cochrane collaboration (Cochrane Review issue 2, 2004).
**Results:** The initial search yielded 1143 studies; after review 27 fulfilled the criteria for inclusion. In 20 studies HbA1c was mentioned. For studies published year <2000 the weighted mean difference (WMD) between SAI-analogues and regular insulin was 0.09% (95% CI:-0.19 to 0.02), whereas for publication year ≥ 2000, the WMD was -0.14% (95%CI: -0.20 to -0.09). Test of heterogeneity yielded p=0.02 for publications year < 2000 and p=0.49 for trials published ≥ 2000.
For the analysis of overall hypoglycaemia 9 studies mentioned the mean frequency of hypoglycaemic episodes per patient per month. For studies published year < 2000 the standardised mean difference (SMD) was 0.01 (95% CI: -0.18 to 0.20) comparing SAI-analogues with regular insulin, whereas for publications year ≥ 2000 the standardised mean difference was -0.22 (95% CI: -0.51 to 0.06).

**Conclusion:** Taking into consideration the low quality of the trials included, the criterion "later publication date" reveals a small but statistically significant improvement in glycaemic control in favour of short acting insulin analogues when compared to regular insulin. Main reasons for the preference of short acting insulin analogues could be an increasing knowledge about the use of analogues and the increasing numbers of parallel study designs in studies published later. The rate of overall hypoglycaemic episodes was not significantly reduced with short acting insulin analogue treatment with respect to publication date.

# PS 76

# Long-acting insulin analogues in Type 1 diabetes

## 841

**Within-person variation in fasting blood glucose is correlated to incidence of hypoglyacemia in people with Type 1 diabetes treated with insulin detemir and NPH insulin**

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**Background and aims:** Hypoglycaemia remains the major barrier to achieving tight glycaemic control in people with diabetes treated with intensive insulin therapy. Previous studies suggested that hypoglycaemia may be closely linked to the high variability observed with traditional basal insulin preparations such as NPH insulin (NPH). We investigated whether a correlation between incidence of hypoglycaemia and within-person variation in fasting blood glucose exists, and whether there is a difference in this correlation between the new soluble basal insulin analogue insulin detemir and NPH insulin.
**Materials and methods:** This meta-analysis included 4 multinational, open-label, randomised phase III trials in people with Type 1 diabetes, treated with a basal-bolus regimen with insulin detemir (n=1336) or NPH insulin (n=814) in combination with pre-meal regular insulin or insulin aspart for 16 weeks up to 6 months. Hypoglycaemia was defined as symptoms +/- blood glucose < 2.8 mmol/L.
**Results:** Subject characteristics were well balanced between treatment groups and representative for the population studied. Comparison of hypoglycaemia incidences demonstrated an estimated reduction by 5.26 episodes per person per year for insulin detemir relative to NPH insulin (insulin detemir: 47, NPH: 52 episodes, p=0.0332). Mean coefficient of variation (CV) for the within-person variation in self-measured fasting blood glucose was lower with insulin detemir than with NPH insulin across trials, (30.9 vs. 33.6%, difference: 2.7%, p=0.001). A clear positive correlation exists between incidence of hypoglycaemia and CV in fasting blood glucose with a slope of 1.02, (p<0.0001), indicating that a difference of 2.7% in the within-person variation in fasting blood glucose, results in 2.77 (≈3) fewer hypoglycaemic episodes per person per year. This correlation is independent of treatment. A similar but less significant relationship was observed for nocturnal hypoglycaemia.
**Conclusion:** The reduction in within-person variability of fasting blood glucose appears to be a major contributor, accounting for about 53%, to the reduced risk of hypoglycaemia observed with insulin detemir relative to NPH insulin.
*The study was sponsored by Novo Nordisk.*

## 842

**Treatment with insulin glargine of patients with Type 1 diabetes in clinical practice: metabolic control over 30 months**

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**Background and aims:** Achieving $HbA_{1c}$ targets of <7% in patients with Type 1 diabetes in clinical practice involves long-term motivation and co-operation by patients and healthcare providers.
**Materials and methods:** Analyses of near-normal weight Type 1 diabetes patients treated with insulin glargine (LANTUS®) over 30 months in combination with a continuous educational programme in clinical practice in Germany are reported. Patients (n=65; 57% male; mean age 40.7 ± 13.3 years; mean body weight 77.0 ± 13.5 kg) were switched from their previous insulin regimen (NPH insulin, n=54; NPH insulin + lente insulin, n=11) to insulin glargine and observed for 30 months. The initial dose of insulin glargine was 80% of the NPH insulin dose and, where relevant, 100% of the lente insulin dose; patients were titrated to a target fasting blood glucose level of 4.5–6.7 mmol/L (80–120 mg/dL). Patients participated in an educational programme prior to insulin glargine initiation and received continuous healthcare advice throughout the study. Metabolic control ($HbA_{1c}$) was monitored at baseline, 9, 18, 24 and 30 months, and change in insulin glargine dose from baseline to endpoint. All variables were analysed using a paired *t*-test, from which mean values and standard deviation were derived.
**Results:** Overall, there was a significant baseline to endpoint decrease in $HbA_{1c}$, which was apparent after 9 months (Table). There was also a

tendency for weight loss throughout the analysed time period. When the results were analysed by previous insulin regimen, a significant improvement in $HbA_{1c}$ was observed in both groups (Table). The insulin glargine dose increased from 19.5 ± 7.8 IU to 21.3 ± 9.1 IU ($p < 0.05$) in patients previously treated with NPH insulin and from 25.4 ± 8.8 IU to 26.5 ± 12.7 IU ($p = 0.4$) in those previously treated with NPH insulin plus lente insulin.
**Conclusion:** This study shows that insulin glargine, in combination with an educational programme, significantly improves metabolic control in patients with Type 1 diabetes in clinical practice.

| $HbA_{1c}$ (%) | All Type 1 (n=65) | Previous regimen | |
|---|---|---|---|
| | | NPH (n=54) | NPH + lente (n=11) |
| Baseline | 7.29 ± 1.1 | 7.27 ± 1.2 | 7.42 ± 1.2 |
| 9 months | 7.20 ± 1.0 | 7.15 ± 0.7 | 7.36 ± 1.8 |
| Change from baseline at 9 months | −0.09; p <0.004 | −0.12; p <0.004 | −0.06; p <0.005 |
| 30 months | 7.06 ± 1.0 | 7.13 ± 1.1 | 6.60 ± 0.3 |
| Change from baseline at 30 months | −0.23; p <0.003 | −0.14; p <0.002 | −0.82; p <0.001 |

## 843

**Decreased hypoglycaemic episodes and less weight gain in patients with Type 1 diabetes transferred from NPH to insulin glargine with maintenance of glycaemic control despite lower insulin doses**
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**Background and aims:** Hypoglycaemic episodes are a significant cause of psychological and physical fear in patients with type 1 diabetes.
**Methods and materials:** An electronic database was used to computer select 196 patients with type 1 diabetes who had comparable baseline $HbA_{1c}$ values. Of the 196 patients, a total of 98 were transferred from NPH insulin to insulin glargine (LANTUS®), and an additional 98 remained on NPH insulin. All patients received intensive therapy, adjusted according to fasting blood glucose levels, with multiple daily injections (MDI) (four/day) in addition to a short-acting insulin (lispro, human regular or aspart) for at least 6 months from April 2001.
**Results:** Baseline demographics for patients treated with insulin glargine or NPH insulin were comparable. Numbers of males and females were similar between groups. Mean age (± standard deviation) was 33.4 ± 10.1 and 31.1 ± 8.7 years in the insulin glargine and NPH insulin groups, respectively ($p = 0.3$). Mean duration of diabetes was 16 ± 10.4 and 16.8 ± 9.2 years, and mean duration of treatment was 13.1 ± 1.1 months and 12.7 ± 0.9 months, for the insulin glargine- and NPH insulin- treated groups, respectively. At the end of the study, mean $HbA_{1c}$ values were not significantly different to baseline between the two groups ($p > 0.05$) (Table). Severe hypoglycaemic episodes were significantly lower in patients treated with insulin glargine compared with patients treated with NPH insulin (0.5 episodes/patient-year and 1.2 episodes/patient-year, respectively [$p < 0.05$]) at the end of the study. Mean total and basal long-acting insulin doses were significantly reduced, compared to pre-transfer doses, from baseline to endpoint in the insulin glargine group, but not in the NPH insulin group ($p < 0.0001$). No change in the short-acting insulin dose was observed from baseline to endpoint in either insulin glargine- or NPH insulin-treated patients ($p > 0.05$). From baseline to endpoint, weight gain was significantly higher in the NPH insulin-treated group compared with the insulin glargine-treated group ($p < 0.05$)
**Conclusion:** In patients with type 1 diabetes, the introduction of intensive MDI insulin glargine treatment leads to sustained glycaemic control, significantly reduces severe hypoglycaemic episodes and is associated with minimal weight gain.

| Group | Total insulin dose (IU/day) | | Basal insulin dose (IU/day) | | Short-acting insulin dose (IU/day) | | $HbA_{1c}$ (%) | | Weight (kg) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline | End | Baseline | End | Baseline | End | Baseline | End | Baseline | End |
| Insulin glargine | 56.6 ± 2.4* | 53.3 ± 2.2 | 38.1 ± 1.8* | 33.2 ± 1.5 | 18.5 ± 0.8 | 20.1 ± 1.0 | 7.4 ± 0.1 | 7.4 ± 0.1 | 75.5 ± 1.5 | 76.0 ± 1.5 |
| NPH insulin | 62.1 ± 2.9 | 62.7 ± 2.6 | 43.3 ± 2.3 | 42.7 ± 1.8 | 18.8 ± 1.2 | 20.0 ± 1.3 | 7.4 ± 0.1 | 7.5 ± 0.1 | 79.5 ± 1.8 | 80.9 ± 1.8 |

*p <0.0001

*Supported by: the Children's Diabetes Foundation, Denver, CO, Barbara Davis Center for Diabetes, Denver, CO, and the Diabetes Endocrine Research Center - NIH, Bethesda, MD.*

## 844

**Lower basal insulin dose requirements, reduction of nocturnal hypoglycemic episodes and improvement of blood glucose control with insulin glargine vs previous NPH treatment in subjects with Type 1 Diabetes**
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**Background and aims:** New therapies for treatment of type 1 diabetes should improve control without increasing the number of hypoglycemic episodes. Previous studies with insulin glargine demonstrated a reduction in the number of episodes of nocturnal hypoglycemia with inconsistent A1 c changes.
**Objective:** To investigate the possibility of achieving A1 c reduction without increasing the frequency of hypoglycemia in a population of typel patients with intensive treatment (basal TID NPH insulin) after being changed to a single bedtime dose of insulin glargine for 1 year.
**Material and Methods:** 30 patients with type 1 diabetes (17 M) mean age of 27.5 ± 14 years and diabetes duration of 10.3 ± 8.4 years, maintained the same treatment for at least one year. Previous treatment consisted in 3 doses of NPH insulin plus pre-prandial short acting insulin analogue (aspart or lispro). Insulin glargine replaced NPH insulin at 75 % of the NPH dose, quarterly visits were maintained
**Results:** A1 c decreased progressively from 7.5 ± 1.2 % to 6.8 ± 0.7 % ($p < 0.01$) at one year of treatment. Mean daily blood glucose decreased from 162 ± 42 to 141 ± 21 mg/dl ($p < 0.05$).During the follow-up, the frequency of mild monthly hypoglycemia during the 1 year of follow-up did not vary (9.9 ± 7.8 vs 9.3 ± 7.2). However, nocturnal hypoglycemia decreased at one year of treatment from 3.8 ± 6.3 to 1.0 ± 1.6 ($p < 0.01$) episodes per month, decreasing at third month of treatment, already significant ($p < 0.01$).Although BMI did not change at all, insulin dose (units/kg) was already decreased after 3 months, [0.72 ± 0.36 vs. 0.60 ± 0.28 ($p < 0.05$)] and remained until the end of follow-up. Frequency of daily home glucose measurements increased from 3 ± 1.7 to 3.4 ± 1.6 ($p < 0.01$).Without severe hypoglycemia or DKA.
**Conclusion:** After transition to insulin glargine, A1 c levels improved and nocturnal hypoglycemia decreased after 1-year follow-up. In contrast to DCCT, BMI did not change and insulin dose was reduced suggesting insulin glargine is the superior basal insulin for type 1 diabetes.

## 845

**Equivalent efficacy of dinner or bedtime administration of insulin glargine combined with regular or fast-acting analogues in Type 1 diabetes**
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**Background and aims:** The aim of this study was to confirm the flexibility of the basal, long-acting insulin analogue insulin glargine (LANTUS®) injection time in patients with Type 1 diabetes using regular insulin (R) or fast-acting analogues (F) as prandial insulin. The primary aims were to demonstrate equivalence in terms of $HbA_{1c}$ at endpoint (delta of equivalence: 0.30%) between insulin glargine administered at dinner (D) versus bedtime (HS) overall and also within the two types of prandial insulin used.

**Materials and methods:** It was a randomized, comparative, parallel, multicentre, open, 6 month study in France. Type 1 patients treated with ≥3 injections/day were switched from their previous basal regimen and randomized to receive once-daily insulin glargine at dinner (6.30–9PM; group D) or bedtime (10PM–12AM; group HS) and continued on their previous prandial bolus regimen: 75% used F and 25% used R. Target fasting blood glucose (FBG) was 80–140 mg/dL; target 2 hour post prandial glucose (2 h PPG) was <160 mg/dL. BG profiles were obtained from 8-point self-monitored BG measurements, the first before switching and 3 others during the last 4 months of the trial, which were averaged to assess the efficacy of the 4 insulin regimens (Table).
**Results:** There were 1178 patients, 900 in the per-protocol analysis set, of whom 446 patients were in group D and 454 in group HS. At endpoint, $HbA_{1c}$ had decreased ($p < 0{,}0001$) by 0.25 ± 0.70% to 7.77 ± 1.0% and by 0.24 ± 0.80% to 7.84 ± 1.10% in the D and HS groups, respectively. The Δ $HbA_{1c}$ D-HS was – 0.022% (two-sided 95% confidence interval [CI; –0.106; 0.063]) demonstrating statistical equivalence between dinner and bedtime insulin glargine administration. This was also demonstrated within the prandial insulin groups: Δ $HbA_{1c}$ was –0.03% in F (two-sided 95% CI [–0.12;0.07]) and –0.04% in R (two-sided 95% CI [–0.22;0.14]). The mean insulin glargine dose was 0.31 ± 0.12 IU/kg/day. The relationship between FBG and $HbA_{1c}$ was confirmed: mean $HbA_{1c}$ in patients with FBG <100 mg/dL, 100–120 mg/dL, 120–140 mg/dL and >140 mg/dL was 7.3% (n=154), 7.5% (n=140), 7.8% (n=157) and 8.1% (n=390), respectively. Considering the prandial insulin action, only the upper limit of the 2 hPP target was reached with F but not with R although R gave a better pre-D BG. The incidence of severe nocturnal and diurnal hypoglycaemia was 0.08 and 0.12 events/patient-year, without any difference between groups.
**Conclusion:** These results show that, in Type 1 diabetic patients treated with a basal-bolus regimen, insulin glargine, plus either F or R, is equally effective and well tolerated, regardless of administration at D or HS. The titration of insulin glargine and prandial insulin to targets allows greater improvement in $HbA_{1c}$.

| 8-point blood glucose mmol/L (mg/dL) | Breakfast | 9AM | Lunch | 2PM | Dinner | 9PM | Bedtime | 3AM |
|---|---|---|---|---|---|---|---|---|
| Before insulin glargine | 9.2 (175) | 10.4 (187) | 7.5 (135) | 8.7 (157) | 8.9 (161) | 9.6 (173) | 9.8 (177) | 8.9 (160) |
| D group | 7.9 (143) | 8.8 (159) | 7.3 (131) | 8.7 (157) | 8.9 (161) | 9.6 (172) | 9.4 (169) | 8.3 (150) |
| HS group | 7.9 (142) | 9.2 (166) | 7.2 (129) | 8.6 (155) | 8.8 (159) | 9.6 (172) | 9.3 (168) | 8.3 (150) |
| Subgroup (n=320) with $HbA_{1c}$ <7.5% at endpoint | 7.2 (130) | 8.0 (144) | 6.7 (121) | 8.4 (151) | 8.0 (144) | 8.5 (153) | 8.2 (147) | 7.8 (140) |

## 846

**Conversion from once- to twice-daily injections of insulin glargine in DM1 patients: comparison by means of a continuous glucose subcutaneous monitoring (*CGMS*™)**

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**Background and aims:** The new insulin analogue Glargine is a valuable tool to obtain a basal insulin supplementation in patients with type 1 diabetes mellitus (DM1). Its pharmacokinetic profile (peakless and long-acting) permits to improve the levels of fasting plasma glucose (FPG) and to reduce the number and intensity of nocturnal hypoglycaemic episodes. Some patients with DM1 under intensified insulin-therapy (one-third of these patients, in our experience), do not reach the glycaemic objectives (FPG, HbA1c, number of hypoglycaemic events,...) despite the titration of both glargine and pre-meals insulins. Frequently, they show high pre-dinner glucose values and hypoglycaemia pre-breakfast. In these cases, we place them on twice daily glargine injections splitting the dose of glargine. Continuous glucose subcutaneous monitoring (CGSM) is a useful method to evaluate glycaemic profiles during over 72 h, offering graphic and numerical data. The aim of this study is to confirm the advantages of conversion from once- to twice daily glargine injections in DM1 patients who do not reach their glycaemic objectives by means of a continuous glucose subcutaneous monitoring (*CGMS*™, *Minimed*™).
**Materials and methods:** A CGSM (*CGMS*™, *Minimed*™) was performed during more than 72 h in 7 consecutive non-selected patients with DM1 inadequately covered with once-daily glargine and pre-meals lyspro injections. After they were placed on a twice-daily glargine injections regime by splitting the dose, the CGSM was repeated. The software *Minimed Solutions 3.0*™ (*Minimed*™) was used in order to obtain graphics and numerical data which were analysed statistically (*SPSS 11.0*™, Wilcoxon Test).
**Results:** (Data are expressed as: mean ± standard deviation once-daily glargine *vs* mean ± standard deviation twice-daily glargine; p) Mean glucose (mg/dl): 165 ± 31.13 *vs* 159 ± 26.41; p = 0.23. Standard deviation (mg/dl): 78.71 ± 13.97 *vs* 65.00 ± 11.69; p = 0.018. Time above high limit (160 mg/dl) (%): 37.43 ± 14.83 *vs* 37.29 ± 14.83; p = 1.0. Time within limits (160–70 mg/dl) (%): 52.14 ± 12.90 *vs* 56.71 ± 18.99; p = 0.67. Time below low limit (70 mg/dl) (%): 10.43 ± 7.59 *vs* 6 ± 8.26; p = 0.10. Mean glucose 1 hour before breakfast (mg/dl): 107.33 ± 44.36 *vs* 135 ± 43.96; p = 0.043. Minimum glucose 1 hour before breakfast (mg/dl): 66.83 ± 32.58 *vs* 104.33 ± 49.5; p = 0.043. Mean glucose 1 hour before dinner (mg/dl): 203.12 ± 50.63 *vs* 182 ± 67.22; p = 0.60.
**Conclusion:** Some patients with DM1 can not reach their glycaemic objectives with once-daily glargine injections. Lower glucose values pre-dinner with lower risk of pre-breakfast hypoglycaemia can be obtained using a twice-daily glargine injections regime by splitting the dose of glargine. CGSM (*CGMS*™, *Minimed*™) is a useful method to study these changes.

## 847

**Equivalence of once-daily and twice-daily insulin glargine administration**

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**Background and aims:** Once-daily insulin glargine is the current basal insulin of choice for patients with type 1 diabetes, but clinical experience has led to the opinion that the activity of insulin glargine wanes in some patients, causing hyperglycemia before the next dose. We tested whether or not twice-daily injection of insulin glargine is superior to once-daily injection for preventing the waning of insulin concentrations at the end of a 24-hour period among patients with type 1 diabetes when identical total daily doses are employed.
**Methods:** Seven subjects with c-peptide negative type 1 diabetes were admitted to the GCRC for two 36-hour studies at least one week apart: age = 35 ± 6 years, duration of diabetes = 18 ± 9 years, HbA1C = 7.5 ± 0.5%, BMI = 31.9 ± 7.8 kg/m$^2$, glargine dose = 36 ± 26 units/day. Patients received full-dose insulin glargine once daily at 0800 and half-dose insulin glargine twice-daily at 0800 and 2000 on successive weeks in random order prior to the two admits. Insulin glargine was held on the day prior to admission to prevent carryover of insulin into the study period, and overnight glucose was stabilized with IV insulin on the evening prior to study. No food or short acting insulin was provided during the study period. Between study hours 20 and 24 (0400–0800 on study day 2), insulin concentrations were assessed every 30 minutes with a super-sensitive assay.
**Results:** During the once-daily study, mean insulin concentrations were reduced after 24 hours compared to mean peak concentrations at 1300 (9.3 ± 7.2 vs. 25.7 ± 15.6 mcU/ml; p=0.02), confirming a waning effect of once-daily glargine. Insulin concentrations for the final four hours of study are shown below. Plasma glucose did not differ between the study conditions during the study: 112 ± 60 vs. 111 ± 67 mg/dl (p=0.93).

| Time Period | Once Daily Glargine | Twice Daily Glargine | P Value |
|---|---|---|---|
| 0400 – 0800 | 12.0 ± 9.8 mcU/ml | 13.1 ± 10.0 mcU/ml | 0.18 |
| 0600 – 0800 | 11.0 ± 8.1 mcU/ml | 11.9 ± 9.1 mcU/ml | 0.39 |
| 0700 – 0800 | 10.3 ± 7.0 mcU/ml | 12.5 ± 10.3 mcU/ml | 0.07 |

**Conclusions:** These data demonstrate that insulin concentrations are equivalent at the end of a 24-hour period when insulin glargine is administered once-daily or twice-daily. These results do not support a role for twice-daily insulin glargine injection.

## 848

**Changing from once- or twice-daily basal insulin to once-daily insulin glargine using two titration regimens in Type 1 diabetes: results from the AT.LANTUS trial**

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**Background and aims:** The traditional intermediate-acting insulin of choice has been NPH insulin, which is commonly used in conjunction with prandial insulins in patients with Type 1 diabetes. However, NPH insulin exhibits a peak of action 4–6 hours following administration and is associated with hypoglycaemia. Further, it does not have a 24-hour duration of action and thus is often administered twice daily. Insulin glargine (LANTUS®), a basal, long-acting insulin analogue, has no pronounced peak and is associated with a 24- hour duration of action. Previous trials have demonstrated insulin glargine to be more effective than NPH insulin with no increased risk of hypoglycaemia.
**Materials and methods:** This was a 24-week, multinational (57 countries), multicentre (409 centres), randomized trial carried out to compare two insulin glargine algorithms (one fixed dose titration algorithm and one variable dose titration algorithm) that were designed to finely adjust the basal insulin to match the physiological requirements to achieve normoglycaemic targets in patients with Type 1 diabetes inadequately controlled on their previous regimen. The titration was based on a target fasting blood glucose (FBG) of 4.4–6.7 mmol/L (80–120 mg/dL). Analysis of the full patient population demonstrated no difference between the two algorithms in terms of endpoint (incidence of severe and other hypoglycaemia, change in $HbA_{1c}$, FBG, insulin dose and body weight; data not shown). The results of the subgroup of patients changing switching from NPH insulin or other intermediate-acting insulin therapy to an insulin glargine-based regimen are reported in this abstract.
**Results:** A total of 2410 patients were treated, of whom 2140 patients completed as per protocol. Of these patients, this subgroup analysed the 1569 patients who changed from NPH insulin (85% of patients) or other intermediate-acting insulin + prandial insulin to basal insulin glargine plus prandial insulin. With insulin glargine, there was a significant baseline to endpoint decrease in $HbA_{1c}$ (from 8.4% to 7.8%; –0.63%; $p < 0.001$) and FBG (from 10.0 mmol/L [179.6 mg/dL] to 6.8 mmol/L [122.5 mg/dL]; $p < 0.001$) in the whole group. From baseline to endpoint, total daily dose of basal insulin increased significantly (from 23.0 IU to 28.8 IU; $p < 0.001$). There was no major change in prandial insulin dose (26.5 IU to 25.8 IU). In this whole subgroup, 8.2% of patients experienced severe hypoglycaemia, with a similar incidence between algorithms (algorithm 1: 8.7% versus algorithm 2: 7.9%).
**Conclusion:** The results from the sub-analysis in 1569 patients with Type 1 diabetes show that changing from NPH insulin or other intermediate-acting insulins, whilst maintaining previous prandial insulin regimens, to the basal long-acting insulin analogue insulin glargine improves glycaemic control in poorly controlled patients. This shows that initiating patients to a more physiological basal insulin and making precise dose adjustments has beneficial effects on $HbA_{1c}$ in diverse clinical settings.
*Supported by: Aventis Pharma.*

## 849

**Quality of life is improved with insulin glargine plus lispro compared with NPH insulin plus regular human insulin in patients with Type 1 diabetes**

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**Background and Aims:** Diabetes has a significant, negative impact on the quality of life (QoL) of patients, thus, the protection and improvement of QoL is an important goal of diabetes care. The Audit of Diabetes-dependent Quality of Life (ADDQoL) is a questionnaire that measures present QoL and average weighted impact (AWI) of diabetes on QoL across 18 individual life domains, including work, social life and enjoyment of food. One of the objectives of this study was to show, using the ADDQoL, that the perceived negative impact of diabetes on QoL is reduced in patients on a combination of insulin glargine (LANTUS®) + insulin lispro (Humalog®), as compared with NPH insulin + regular human insulin.
**Materials and Methods:** This was a 32-week, multicenter, open-label, randomized crossover clinical trial comparing once-daily insulin glargine + insulin lispro with once- or twice-daily NPH insulin + regular human insulin, in patients with Type 1 diabetes. Patients (n=48; 62.5 % female; mean age 42 ± 11.4 years) were randomized to receive either Treatment Sequence A (insulin glargine + lispro followed by NPH insulin + regular human insulin; n=22) or Treatment Sequence B (NPH insulin + regular human insulin followed by insulin glargine + lispro; n=26). The ADDQoL was used initially, and after each treatment period. In addition, treatment satisfaction was assessed using the Diabetes Treatment Satisfaction Questionnaire (DTSQ).
**Results:** For all patients combined, the mean present QoL baseline score was 1.3, reflecting 'good' (rather than 'very good' or 'excellent') present QoL. Present QoL improved significantly with insulin glargine + lispro but did not change with NPH insulin + regular human insulin ($p=0.014$; 95% confidence interval [CI]: 0.07; 0.55; Table). The mean AWI score was –1.8 initially, indicating a mean negative impact (actual range –6 to +3) of diabetes on QoL. After the total treatment period, the mean AWI score improved by 0.4 with insulin glargine + lispro and by 0.1 after NPH insulin + regular human insulin, indicating a beneficial effect of insulin glargine + lispro ($p=0.033$). Moreover, treatment satisfaction for the total treatment period was markedly improved after treatment with insulin glargine + lispro compared with NPH insulin + regular human insulin (mean: 32.2 ± 3.4 vs 23.9 ± 7.2; $p<0.0001$).
**Conclusion:** This study shows that insulin glargine + lispro improves treatment satisfaction, reduces the negative impact of diabetes on QoL, and improves QoL *per se*.

| ADDQoL scores (mean ± SD) | Present QoL | | Average weighted impact score | |
|---|---|---|---|---|
| | Sequence A: insulin glargine + lispro/NPH insulin + regular | Sequence B: NPH insulin + regular/insulin glargine + lispro | Sequence A: insulin glargine + lispro/NPH insulin + regular | Sequence B: NPH insulin + regular/insulin glargine + lispro |
| Baseline | 1.4 ± 0.8 | 1.2 ± 1.3 | –1.6 ± 1.2 | –1.9 ± 1.3 |
| End of treatment period 1 | 1.6 ± 0.8 | 1.2 ± 1.2 | –1.2 ± 0.6 | –1.8 ± 1.2 |
| End of treatment period 2 | 1.3 ± 0.8 | 1.6 ± 0.9 | –1.5 ± 1.2 | –1.6 ± 1.2 |
| | **Insulin glargine + lispro** | **NPH insulin + regular** | **Insulin glargine + lispro** | **NPH insulin + regular** |
| Total treatment period | 1.6 ± 0.8* | 1.3 ± 1.0 | –1.4 ± 0.9† | –1.7 ± 1.2 |

*$p=0.014$ vs NPH insulin + regular; †$p=0.033$ vs NPH insulin + regular
*Supported by: Aventis Pharma.*

## 850

**Decreased, nocturnal glycemic variation with insulin glargine compared to insulin ultralente in a randomized, crossover clinical trial**

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**Background and aims:** Multiple daily insulin injection programs achieve glycemic control in type 1 diabetes with significant glycemic variation and hypoglycemia. We measured glycemic excursions utilizing the Continuous Glucose Monitoring system (CGMS® Medtronic MiniMed) in a randomized, 2-period crossover clinical trial to evaluate use of Insulin Glargine (Glargine) versus Insulin Ultralente (Ultralente) as basal insulin replacement when given as a single subcutaneous bedtime injection. We hypothesized that glycemic excursions with use of Glargine would be reduced when compared to Ultralente.
**Materials and methods:** CGMS was utilized at baseline and at the end of each 4-month treatment period to measure glycemic variability in 22 individuals (44 ± 14 years, 55% male, baseline A1c <7.8 %). Average glucose concentration was similar with both treatments in a three-day period ($p=0.153$).

**Results:** The mean glucose over three days with Glargine and Ultralente was 138.4 and 148.2 mg/dl, respectively. The standard deviation (SD) of CGMS sensor values over the three day period of measurement tended to be higher (p=0.070) with Ultralente (66.07) than Glargine (59.05). Median nighttime (11pm–6am) glucose concentrations were not different with the two treatments. The SD of CGMS sensor values during the night was lower (p=0.042) with Glargine than Ultralente. Average 2 hours post-prandial CGMS sensor values and SD of those values were not different over the 3-day period (p=0.3118 and 0.1140 respectively). Percentage of sensor values <70, 70–140, and >140 over a 3-day period (13.62 ± 10.46 vs. 11.93 ± 7.87, 44.31 ± 13.25 vs. 43.05 ± 15.22, 42.08 ± 16.43 vs. 45.01 ± 16.84%, Glargine vs Ultralente p=0.956, 0.133, 0.475 respectively) was not different between the two treatment arms.
**Conclusion:** We conclude that, whereas overall variability is similar when Glargine and Ultralente are used as basal insulin in people with type 1 diabetes, Glargine resulted in less variable nocturnal glucose values, suggesting more consistent absorption during the first twelve hours after subcutaneous injection.

## 851

**Significant reduction in blood glucose variability with insulin detemir versus NPH insulin: Confirmed by meta-analysis of continuous glucose monitoring**

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**Background and aims:** Clinical trials have consistently shown glycaemic control with insulin detemir (IDet) to be at least as effective as NPH insulin in people with diabetes, with a lower risk of nocturnal hypoglycaemia and lower within-subject variation in fasting blood glucose (BG). To investigate whether insulin detemir is associated with less fluctuation in BG levels than NPH insulin, a meta-analysis of 24 h continuous BG profiles from 5 phase III trials in people with Type 1 and Type 2 diabetes was performed.
**Materials and methods:** All trials were multinational, randomised, open-label, parallel group trials comparing basal-bolus therapy with insulin detemir or NPH insulin in combination with regular insulin or insulin aspart with meals over 4 to 6 months. For ethical and practical reasons, a subgroup of subjects in each trial (Type 1: IDet = 393, NPH = 197; and Type 2: IDet = 112, NPH = 56) were asked (but not required) to wear the MiniMed Continuous Glucose Monitoring System (CGMS) for 72 h during the last month of treatment. Baseline demographic characteristics were well balanced between treatments, and were generally representative of the respective patient populations. Variability in BG levels was expressed by estimation of fluctuations and excursions with each treatment. Fluctuation was defined as the area between the BG curve and the subjects individual average BG level from 23:00 to 06:00 (nocturnal), or over 24 h. Similarly, excursion was defined as the area where BG was outside the desired BG range 4–10 mmol/L. Fluctuations and excursions were analysed across trials in a repeated measures ANOVA with treatment and trial as fixed effects.
**Results:** BG levels fluctuated less both nocturnally and over 24 h with insulin detemir than with NPH insulin. The extent of BG excursions below < 4 mmol/L was significantly lower nocturnally for subjects treated with insulin detemir.

Variability in 24 h Continuous BG Profiles (mmol*h)

| | IDet mean | NPH mean | IDet-NPH mead diff. 95% CI | p-value |
|---|---|---|---|---|
| **Nocturnal fluctuation** | 8.79 | 9.97 | -1.18 [-1.86; -0.51] | <0.01 |
| excursion <4 mmol/L | 0.82 | 1.08 | -0.25 [-0.49; -0.01] | 0.04 |
| excursion >10 mmol/L | 4.41 | 4.82 | -0.42 [-1.46; 0.63] | 0.44 |
| **24h fluctuation** | 48.73 | 53.74 | -5.01 [-8.15; -1.87] | <0.01 |
| excursion <4 mmol/L | 2.15 | 2.63 | -0.48 [-1.02; 0.05] | 0.08 |
| excursion >10 mmol/L | 12.99 | 15.17 | -2.18 [-5.06; 0.70] | 0.14 |

**Conclusion:** These findings demonstrate that insulin detemir produces a flatter and smoother time action profile than NPH insulin in a basal-bolus regimen. The observed reduction in nocturnal BG excursions below 4 mmol/L is consistent with the observed lower risk of nocturnal hypoglycaemia.
*The Studies were sponsored by Novo Nordisk.*

# PS 77
# Devices in clinical practice

## 852

**Effect of a pocket-size tablet dispensing device on glycemic control in Type 2 diabetic patients**

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**Background and aims:** It has been shown repeatedly that adherence to oral medication is low in type II diabetic patients, especially in a regimen with tablet administration more than once daily, thus diminishing therapeutical effect. On the other hand HbA1 c reduction as little as 0.2% has been shown to significantly reduce the risk for cardiovascular mortality. The aim of this study was to evaluate whether a pocket-size tablet dispensing device would improve adherence to therapy as evidenced by reduction of HbA1 c levels in a large population of type II diabetics.
**Materials and methods:** The study design was prospective, randomized, open label with two parallel groups. 2296 patients were recruited from general practitioners and internists and randomized to either receive a tablet dispenser (TD) small enough to be carried around and especially designed for the needs of diabetic patients or no intervention (control group, CO). Patients characteristics (height, weight, age, gender, blood pressure (RR)) and current oral therapy (including dosage) were recorded at baseline. HbA1 c was measured at baseline and after 3 and 6 months intervention at normal control visits. Changes in HbA1 c levels were compared between the 2 groups. Results are presented as mean [95% confidence interval]
**Results:** Only 19 patients were lost to follow-up. After exclusions of additional case report forms (CRFs) for varying reasons (e. g. insulin therapy) 2081 CRFs were evaluated. At baseline, age, BMI and gender distribution were comparable between the two groups, as well as HbA1 c (7.99 [7.91–8.08], TD vs. 7.97 [7.89–8.05], CO). In contrast, RR was significantly lower in the TD group. After 6 months, HbA1 c improved in both groups, but improvement was significantly greater in TD than in controls (–0.73 [0.67 – 0.80] vs. –0.53 [0.47 – 0.59]). Subgroup analysis revealed that the effect was significantly more pronounced 1) in patients receiving ≥ 5 tablets/day than in patients with < 5 tablets and 2) in patients < 55 yrs than in patients ≥ 55 yrs.
**Conclusion:** In this large study population in a „realistic" setting a simple tablet dispensing device led to a significant and relevant improvement in HbA1 c levels. The fact that the benefit was greater for younger patients and those with a more complicated therapy regimen suggests that the effect was due to improved adherence to therapy.

## 853

**Glucometer-based postprandial glycemia determination allowing alternative sites blood collection in clinical practice**

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**Background and aims:** Increased use of glucometers allowing blood collection from alternative sites has led to increased attention given to the so-called "AST-like phenomenon" referring to different glycemia determinations when collecting blood from an alternative sites compared with fingertip blood collection in the presence of rapid changes in blood sugar levels. This phenomenon is regarded as one associated with risk in the case of hypoglycemia. Papers published to date have largely described this phenomenon in experiment following intravenous glucose administration. In practice, similar rapid changes occur, e. g., following food intake. Our study was designed to test the reliability of blood sugar determination using a glucometer and a blood sample obtained by collection from an alternative site compared with simultaneous fingertip determination and a control laboratory determination.
**Materials and methods:** We made 200 blood collections to determine blood sugar levels in patients hospitalized at the Diabetes Center, Institute for Clinical and Experimental Medicine. Blood collections were made before a meal (n=50), with a paired collection 1 hour after the meal (n=50). Two more collections were made at the same time: alternative site collection (forearm; n=100) and control fingertip collection (n=100). The blood sample obtained from an alternative site was examined using the glucometer whereas the fingertip blood sample was analyzed using the glucometer and the laboratory (FreeStyle, TheraSense and Beckman Analyser, Beckman

Instruments, USA). Statistical analysis was undertaken using regression analysis and the *t*-test. Further, we determined the number of qualifications exceeding the admissible tolerance of error of measurements (Bland-Altman method). Results are expressed as the number of determinations in excess of the mean ± 2SD (SD = standard deviation) of the values.
**Results:**

The method of linear regression revealed the following correlations:

| | n | r Forearm on glucometer vs fingertip on glucometer | r Forearm on glucometer vs fingertip in laboratory | r Fingertip on glucometer vs fingertip in laboratory |
|---|---|---|---|---|
| Preprandial | 5 | 0.980 | 0.983 | 0.990 |
| determination | 0 | | 0.982 | 0.990 |
| Postprandial | 5 | 0.987 | | |
| determination | 0 | | | |

A comparison of results obtained from fingertip and forearm glucometer determinations with corresponding fingertip laboratory determinations using the paired *t*-test revealed a significant difference between forearm determination at 1 hour postprandially vs fingertip laboratory determination at 1 hour postprandially ($p<0,01$). No difference was observed when determining preprandial glycemia or postprandial glycemia as determined using only the glucometer. None of the compared determinations departed significantly from the allowable deviation defined as the mean ± 2SD of the values measured.
**Conclusion:** Our results did not demonstrate clinically significant deviations in postprandial glycemia determinations associated with alternative site blood collection. Major deviations in postprandial versus laboratory measurements were associated with glycemia levels over 22 mmol/l.
*Supported by: VZ/CEZ:L17/98:00023001*

## 854

**Bolus pre-prandial vs. bolus post-prandial to correct abnormal glucose values in Type 1 diabetics treated with continuous subcutaneous insulin infusion (CSII) evaluated by means of the OneTouch®UltraSmart™ Blood Glucose Monitoring System (US-BGMS)**
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**Background and aims:** Pre-prandial corrector bolus (Pre-B) were used by pump patients to adjust insulin doses to elevated or reduced glucose values before meals. If the use of a corrector bolus to elevated post-prandial glucose values (post-prandial corrector bolus, Post-B) is better than Pre-B is not known. The aim of the study was to compare Pre-B vs. Post-B to correct abnormal glucose values in type 1 pump diabetic patients.
**Materials and methods:** In this prospective, randomised, open cross-over study, 16 type 1 diabetics (age 32.5 ± 8.5 years-old, 14 women, BMI 22.3 ± 2.0 kg/m$^2$, diabetes duration 17.6 ± 8.3 y, under CSII since 2.2 ± 0.8 y, lispro insulin dose 0.55 ± 0.10 U/kg/d, as basal 51 ± 9%, $HbA_{1c}$ 6.9 ± 0.7%) were randomised to a treatment with Pre-B or Post-B followed by the alternative treatment, each for 2 weeks. Pre-B were calculated based on pre-meal glucose values: (BG-100 mg/dl)/SF (individual sensitivity factor) and Post-B on 1.5 h post-meal glucose values: (BG-140 mg/dl)/SF. Apart of corrector bolus, all patients adjusted the prandial bolus according to the number of carbohydrate units (carbs). Mean daily glucose values, pre- and post-prandial glucose values, glycemic excursions, variability of glucose profile, Pre-B, Post-B and hypoglycemia events were registered by means of US-BGMS. All statistical analyses were performed using SPSS software. Results are presented as means ± SD. Statistical evaluations were carried out by Wilcoxon test for paired data with a significance level of $< 0.05$.
**Results:** Only US-BGMS data from 14 patients were suitable for analysis. Mean daily and 1.5 h post-prandial glucose values were lower with Pre-B (Pre-B vs. Post-B, 158.6 ± 20.3 vs. 168.1 ± 19.1 mg/dl and 164.7 ± 23.8 vs. 192.2 ± 26.5 mg/dl, respectively, both $p < 0.05$) but not pre-prandial or nocturnal (3 AM) glucose values. Glycemic excursions were also lower with Pre-B 16.7 ± 33.4 vs. 48.4 ± 33.5 mg/dl ($p < 0.05$) as well as the variability of glucose profile 61.8 ± 9.9 mg/dl vs. 70.6 ± 16.7 mg/dl ($p < 0.05$). No differences were found in the number of carbs consumed, prandial bolus or corrector bolus (either Pre-B or Post-B) for each mean meal. The number of total hypoglycemic events, severe hypoglycemia (< 55 mg/dl) or symptomatic hypoglycemia were similar during both periods.
**Conclusion:** Pre-B was better than Post-B and remained the best approach to correct abnormal values in type 1 diabetic pump patients. Pre-B achieved lower post-prandial glucose values, minor glucose excursions, better mean daily glucose profile and lower glucose variability with no increment in insulin corrector doses or hypoglycemia.

## 855

**Continuous glucose monitoring for 5 days using a novel glucose-electrode in patients with Type 1 diabetes**
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**Background and aims:** To evaluate efficacy and safety of a functional model of the iSense system for continuous glucose monitoring under close to daily life conditions in comparison to a system which is already on the market.
**Materials and methods:** Fifteen patients with type 1 diabetes (age 43 ± 10 years; BMI 24.9 ± 2.7 kg/m$^2$ (mean±SD)) were enrolled in this open, single-center study. Each patient participated in one experiment with continuous sensor recordings over 5 days. On the first day, the iSense sensor and a CGMS sensor (Medtronic MiniMed, Gold version, Düsseldorf, Germany) were placed into the abdominal subcutaneous fat tissue of the patients on opposite sites. The patients remained in-house throughout the study and kept insulin therapy and diet as usual. Both glucose sensors consisted of a flexible microelectrode with a thin coating of glucose oxidase beneath several layers of biocompatible membranes. Insertion is achieved by means of a rigid introducer needle which, after sensor placement, is automatically retracted in case of the iSense and manually removed in case of the CGMS. According to the manufacturer recommendations the CGMS sensor was replaced by a fresh sensor after 3 days. In contrast, the iSense sensor remained in place for the entire experiment. Twenty capillary blood glucose (BG) measurements per day - performed in duplicate by means of a blood glucose meter (Accu-Chek Active; Roche Diagnostics, Mannheim, Germany) – were used as reference points for sensor evaluation. Linear regression over the variables sensor current and reference BG enabled calculation of predicted BG values for both glucose monitoring systems. From the paired data sets the deviations between reference BG values vs corresponding predicted values were determined. This approach allowed calculation of various assessment parameters.
**Results:** The error grid analysis showed that for the iSense sensor 95.2% and the CGMS 97.9% of all paired data points fell into zones A and B (n.s.). The MAD (median of the absolute values of the percent deviations) (14.5 ± 5.0 vs. 11.7 ± 4.1, n.s.) and the %PRESS (percent predicted residual sum of squares) values (25.2 ± 8.7 vs. 15.6 ± 4.2, n.s.) were also comparable. The system error (percent deviations <= 50 taken and 95.5% of these points located between the boundaries (+) and (–) system error) of all measurements was comparable (35.0 ± 8.1 vs. 30.1 ± 7.6, n.s.; iSense vs. CGMS). Also the drift of the sensor signals (i.e., the slope of the regression line vs. time of the percent deviations) for the iSense sensor and the CGMS was comparable (–6.6 ± 7.2 vs. –5.6 ± 9.2 %/day, n.s.). Three CGMS sensors and two iSense sensors had to be replaced due to technical failures; 5 iSense sensors did not automatically retract needle after application. No other sensor related side effects were observed.
**Conclusion:** This study demonstrated that the iSense prototype system showed a comparable performance to that of an established glucose monitoring system. Superior properties of the iSense system are the long duration of application (5 days) and – in its final version – the need for maximal two calibrations per day only.

## 856

**Pilot study on the effect of disturbed microvascular skin blood flow on glucose measurements obtained from the lower forearm during dynamic blood glucose changes**
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**Background and aims:** Measurement of blood glucose derived from skin areas different from the fingertip were shown to provide some discordant results, especially during fast glucose dynamics. Although the exact mechanism behind this phenomenon is still unknown, it is assumed that anatomical differences in skin microcirculation might be involved.
**Materials and methods:** In our study, 12 type 1 diabetic patients (5 female, 7 male; age 33.3; 23–53 years; duration of diabetes 10.8; 1–27 years; HbA1c 6.6 %; 6.1–7.3 %; mean, range) received an oral glucose load with subse-

quent blood glucose and skin microcirculatory measurements. Patients ingested 75 g of liquid glucose and after 2 hours, insulin was applied intravenously to achieve a rapid blood glucose decline. Blood glucose was measured every 15 min at the fingertip (Super GL, Analyser, Müller Gerätebau) and at the lower forearm (SoftSense, Abbott MediSense) for a total duration of 4 h. In addition, skin microvascular blood flow was measured by laser-dopplerfluxmetry at the lower forearm (LDF, MBF 3D, Moor Instruments).
**Results:** During increasing blood glucose levels, no significant differences could be observed between glucose results measured at the lower forearm and those obtained from the fingertip. During seceding blood glucose levels, a significant time shift in glucose measurements could be observed between AST and the measurement from the fingertip ($p<0.001$, respectively). After intravenous application of insulin a significant increase in skin microvascular blood flow could be measured by LDF ($p<0.05$). When our study group was subdivided in patients without significant deviation in blood glucose values <30% (Non-Deviators) and those showing a deviation ≥30% (Deviators), deviators showed a significantly impaired microvascular response to insulin at the lower forearm (AUC 834.4 ± 306.5 vs. 139.2 ± 62.8 arbitrary units; mean ± SEM, $p<0.05$).
**Conclusion:** This is the first study to suggest, that patients showing deviations in blood glucose measurements at different skin sites following application of insulin might be identified by a decreased skin microvascular reactivity to insulin.

## 857

**Continuous glucose monitoring for the evaluation of peri-operative stress in patients with Type 2 diabetes, impaired glucose tollerance and normal subjects**

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**Background and aims:** Peri-operative stress is known to be associated with an increase of counter-regulatory hormones which may increase an increase the blood glucose levels. The aim of this study was to evaluate the changes occurring in blood glucose *during peri-operative stress* by using the Continuous Glucose Monitoring System (CGMS) in patients with type 2 diabetes and impaired glucose tollerance (IGT).
**Patients and methods:** Eight patients affected by type 2 diabetes (4M, 4F; mean age 58 ± 11yrs.; BMI=25,3 ± 3; mean disease duration=15.5 ± 11yrs.), five IGT patients (4 M, 5 F; mean age 55,8 ± 11 yrs., BMI=26 ± 3.3) and nine normal subjects (4M, 5F; mean age 47.2 ± 15.9 yrs., BMI=25.1 ± 2.9) were studied. All subjects underwent general anaesthesia for elective non abdominal surgery.
All patients received insulin therapy according to the recognized protocol for surgery in diabetic patients. CGMS was inserted in peri-umbelical region the day before surgery and removed one day postoperative for total monitoring of 48 hrs monitoring (288 lecture for each day). Data were recorded by a computer as numerical values and transformed into daily graphs.
**Results:** Mean glycaemic peak during all monitoring period was 253,6 ± 75 mg/dl in type 2 diabetic patients, 154,4 ± 44 mg/dl in IGT patients and 131,8 ± 27 mg/dl in normal subjects. In pre-operative period, mean glycaemic peak was 187 mg/dl in type 2 diabetic patients, 150 mg/dl in IGT patients and 109 mg/dl in normal subjects; during surgery mean glycaemic peak was 248 mg/dl in type 2 diabetic patients, 148 mg/dl in IGT patients and 114 mg/dl in normal subjects; in postoperative period mean glycaemic peak was 224,6 mg/dl in type 2 diabetic patients, 124 mg/dl in IGT patients and 116 mg/dl in normal subjects.
In all diabetic patients raised BG levels (over 200 mg/dl) were observed before, during or after surgery; four patients exceeded 250 mg/dl. Two IGT patients showed an increase of BG levels (>180 mg/dl) during and 6 hours before surgery.
In two normal subjects an increase of BG levels (untill 180 mg/dl) was observed during and 4 hours after surgery; overall peak BG levels never exceeded 110 mg/dl.
**Conclusion:** These data indicate that BG levels can be abnormally high in patients with type 2 diabetes and IGT undergoing surgery, probably in relation with the pre-operative stress or post-operative pain. Interestingly, an increase in BG levels can occur also in subjects with normal glucose tolerance and without family history for diabetes undergoing the stress of surgery, *thus possibly identifying* individuals at risk of developing type 2 diabetes. CGMS can be easily applied to detect such changes.

## 858

**Early detection of hyper- and hypoglycemic excursions in subjects with Type 1 diabetes using a long-term continuous glucose sensor**

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**Background and aims:** A feasibility study was conducted in 10 patients with type 1 diabetes using a second-generation, long-term implantable glucose sensor (DexCom, San Diego, CA).
**Materials and methods:** Of the 10 patients initially implanted, 3 patients were excluded or explanted early due to poor function of the sensor biointerface. The remaining 7 patients were implanted for 119–133 days during which they were first blinded to the continuous data (for 62 ± 6 days), and then unblinded (65 ± 7 days). Data was compared to a One Touch Ultra Meter (Lifescan, Milpitas, CA) to evaluate clinical accuracy. The amount of time spent in glucose ranges and the duration and frequency of hyper and hypoglycemic excursions occuring in the blinded and unblinded phases were analyzed. Hyper and Hypoglycemic excursions were defined as sensor glucose values > 200 mg/dl or < 80 mg/dl that persisted for at least one hour.
**Results:** Of the 1,620 coincident meter and sensor glucose values collected from these patients across both study phases, 90.1% of points fell in the A&B regions of the Clarke Error Grid, 6.79% of points fell in the D&E regions. After unblinding, patients spent a median (interquartile range) of 2.22 (0.46–2.70) fewer hours/day ≥ 240 mg/dl (intrapatient difference, one-sided Wilcoxon signed-rank test, $p = 0.038$), 2.26 (1.31–2.72) more hours/day in the target range of 80–140 mg/dl ($p = 0.26$), and 0.27 (0.05–0.72) fewer hours/day ≤ 55 mg/dl ($p = 0.075$) compared to when blinded to the continuous glucose data. The mean number of hyper and hypoglycemic excursions per day did not change after unblinding (Table 1). However, after unblinding, mean hyperglycemic excursion duration was reduced by 92 minutes (two-sided two-sample paired t-Test, $p = 0.05$), and hypoglycemic excursion duration was reduced by 43 minutes ($p = 0.003$).
**Conclusion:** These results suggest that long-term continuous glucose information can facilitate diabetes management by improving patient awareness and response to glucose excursions.

Table 1

| | Mean ± SEM excursions per day | Mean ± SEM excursions per day | Mean ± SEM excursion duration | Mean ± SEM excursion duration |
|---|---|---|---|---|
| | *Blinded Phase* | *Unblinded Phase* | *Blinded Phase* | *Unblinded Phase* |
| **Hyperglycemic Excursions (≥ 200mg/dl)** | 1.36 ± 0.23 | 1.48 ± 0.33 | 307 ± 62 | 215 ± 29 |
| **Hypoglycemic Excursions (≤ 80mg/dl)** | 1.32 ± 0.23 | 1.32 ± 0.26 | 181 ± 15 | 138 ± 10 |

## 859

**Interfering effects of paracetamol (acetaminophen) and uric acid on glucose meters**

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**Background and aims:** Elevated concentrations of medications and blood constituents may interfere with the measurement by glucose meters. We studied the effects of two substances – paracetamol (also known as acetaminophen, commonly found in pain-relief medications) and uric acid (may be found at elevated levels in renal diseases) – on two glucose-monitoring systems.
**Materials and methods:** Pre-breakfast venous blood samples were collected from healthy subjects who had not taken any medications for at least 24 hours before blood collection. Two to four hours after collection, the blood samples had glucose concentrations between 3.1 and 3.7 mmol/L (56–67 mg/dL) and were used in the studies. In the dose-response study, varying concentrations (up to 14 mg/dL) of paracetamol or uric acid were added to aliquots of the venous blood sample and then each aliquot was

tested 10 times with two glucose monitoring systems based on electrochemical technology. Meter A uses glucose oxidase on the test strip, which requires 1 microliter of blood and 5 seconds for a test. Meter B uses glucose dehydrogenase (GDH-NAD) on the test strip, requiring 1.5 microliter of blood and 10 seconds for a test. In the additive-effect study, paracetamol (4 mg/dL) and uric acid (9 mg/dL), alone and in combination, were added to aliquots of the venous blood sample and then each aliquot was tested 10 times with the two monitoring systems. Results were compared to the control blood aliquot with no substance added. The selected concentrations of those substances were based on published reports of peak plasma paracetamol concentrations up to 4 mg/dL after therapeutic doses and plasma uric acid concentrations above 9 mg/dL in renal failure.
**Results:** In the dose-response study, results of Meter A increased as a function of the concentrations of paracetamol and uric acid, with maximum biases of 61% and 59%, respectively. Results of Meter B showed clinical insignificant changes (maximum biases of less than 3%). In the additive-effect study, paracetamol (4 mg/dL), uric acid (9 mg/dL) and their combination caused significant biases ($p<0.001$) of 12%, 31% and 41%, respectively with the Meter A. Addition of paracetamol, uric acid and their combination to a hypoglycemic blood sample (3.6 mmol/L; 65 mg/dL) caused Meter A to report euglycemic values of 4.0, 4.7 and 5.1 mmol/L (72, 85 and 91 mg/dL), respectively. These substances produced no significant changes (biases < 2%; $p>0.1$) on Meter B results.
**Conclusion:** We found that paracetamol (at a level equivalent to the peak plasma concentration after therapeutic doses) and uric acid (at an elevated level found in renal diseases) each caused falsely high results with one brand of glucose meter. Paracetamol and uric acid together resulted in an additive effect and produced a greater interference. Paracetamol and uric acid did not produce any clinically significant biases in the results from another brand of glucose meter. Health care providers and patients need to be aware of the limitations of their glucose meters.

## 860

**Reuse of insulin syringes**
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**Objective:** BD medical (Becton and Dickinson Company) has published a picture series showing insulin syringes after 1, 3, and 5 injections. We and our patients were astonished how deformed the tips of the syringes looked after only a few injections.
**Materials and methods:** One physician, two nurses all ordinary built and without scars or metal implants. The nurses tested four different syringes each, and used the syringes 1, 3, 5, and 10 times, respectively, for injections in the abdominal fat. One physician used one syringe and injected 1, 3, and 5 times in the abdominal fat. After that the syringe was cut into an ordinary rubber mouse-pad 100 times, and then stabbed into a wooden computer table, and finally into a metal lamp foot. Pictures were taken from the syringes with a Zeiss Axioscope 2.5 × lens with a lateral light source and a Canon 10 D digital camera with 6.2 mpx resolution.
**Results:** The only syringe that looked something like the used syringes in BD's picture series was the one that was cut into a metal lamp foot. The syringes used 0, 1, 3, 5, 10, and 100 times were without any distortion of the tip.
**Conclusion:** Either BD has improved the quality of their insulin syringes dramatically since the pictures were published or BD has manipulated the syringes to produce false evidence of syringe defects.

# PS 78
# Alternative insulin delivery

## 861

**The availability of inhaled insulin leads to improved health outcomes in patients with uncontrolled Type 2 diabetes**
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**Background and aims:** Exubera®, a dry-powder inhaled insulin, is being developed by Pfizer Inc and Aventis Pharmaceuticals Inc in conjunction with Nektar Therapeutics. To evaluate the potential health outcomes with the availability of Exubera® in patients with type 2 diabetes, we modeled physiological outcomes, complications (microvascular and macrovascular), and mortality based on diabetes treatment choice from a randomized trial.
**Materials and methods:** We simulated 100,000 (10,000 × 10) patients based on diabetes treatment choice from a randomized trial in 7 countries (Can, Fra, Ger, Ita, Esp, Swe, USA) in patients with type 2 diabetes failing on diet or oral therapy ($HbA_{1c} \geq 8\%$). Subjects were randomized either to a medical setting where all available diabetes treatment options and Exubera® were available or to a setting where only currently available treatments were available. Subjects with their physician/diabetes nurse made a choice of diabetes therapy, including no change in their current treatment and completed a questionnaire concerning their choice. Diabetes medication choice in a medical setting where Exubera® was available was: continue oral agent therapy (29%), injected insulin (8%), Exubera® (35%), no change in therapy (28%). In a setting with only currently available treatment choices were: continue oral agent therapy (56%), injected insulin (15%), no change in therapy (29%). Health outcomes were simulated using the Economic Assessment of Glycemic control and Long-term Effects (EAGLE) model, which assesses the influence of multiple parameters on the outcomes of diabetes patients over the course of 1 to 10 years. Outcomes predicted included short-term effects (eg, hypoglycemia) and long-term outcomes (eg, micro- and macro-vascular complications). $HbA_{1c}$ is the main influence parameter.
**Results:** Type 2 patients characteristics at baseline were 60% male/40% female, mean duration of diabetes 14 years, mean age 56.7, and $HbA_{1c}$ 9.3%. After 10 years, patients treated in a medical setting where Exubera® was available showed improvements in glycemic control and had an absolute 1.2% lower $HbA_{1c}$ relative to patients with only currently available treatments (8.4% vs 9.8%). Differences between the two groups in $HbA_{1c}$ occurred every year from year 1 to year 10 with lower $HbA_{1c}$ in the Exubera® setting. Cumulative incidence of severe hypoglycemia after 10 years per 100 subjects in the Exubera® setting vs current therapy setting was 7.7 vs 5.4, respectively. Cumulative complications were lower in the Exubera® setting versus current therapy setting by 11% for microvascular events (272.2 vs 305.7 cumulative events per 100 subjects), 8% for macrovascular events (18.1 vs 19.6 cumulative events per 100 subjects), and 6% for mortality (37 vs 39.5 cumulative events per 100 subjects).
**Conclusion:** In patients with type 2 diabetes with inadequate control despite diet or oral agent therapy, the availability of Exubera® as a potential treatment option may improve short- and long-term health outcomes.

## 862

**Inhaled insulin leads to a greater potential acceptance of insulin therapy in patients with uncontrolled Type 2 diabetes**
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**Background and aims:** Many physicians and patients are reluctant to use insulin therapy because of the need for regular injections. Exubera®, a

dry-powder inhaled insulin, is being developed by Pfizer Inc and Aventis Pharmaceuticals Inc in conjunction with Nektar Therapeutics. Preliminary studies have shown that Exubera® provides reproducible and effective control of meal-related glycemia without the need for an injection. We examined the impact of its potential availability on patient acceptance of insulin.
**Materials and methods:** Patients with type 2 diabetes failing on diet or oral therapy ($HbA_{1c} \geq 8\%$) were randomized to 2 groups. Both groups (A and B) received educational information on currently available treatment options; Group B also received educational material about Exubera® as another potential treatment. Subjects were then asked to make a choice of diabetes therapy, including no change in their current treatment. Patients consulted with their physician/diabetes nurse on treatments and completed questionnaires concerning their choice. The primary outcome was the proportion of patients choosing insulin.
**Results:** In total, 779 patients (A=388, B=391) were recruited in 7 countries (Can, Fra, Ger, Ita, Esp, Swe, USA). In Group B, 169 patients (43.2%) chose a treatment option that included insulin compared with 60 (15.5%) in Group A (odds ratio 4.16, 95% CI [2.93, 5.95]; $P < 0.0001$). Despite a mean $HbA_{1c}$ of 9.1%, 168 patients (43.3%) in Group A chose to make no change to their therapy. Significantly fewer Group B patients (107 [27.4%]; odds ratio 0.49, 95% CI [0.36, 0.67], $P < 0.0001$) chose to make no change. Amongst options in Group B, Exubera® was most frequently chosen (35.3%). No systematic differences between countries were found.
**Conclusion:** In type 2 diabetes patients with inadequate control despite diet or oral therapy, the availability of Exubera® as a potential treatment option gets more patients on insulin (43.2% vs 15.5%). The result of Exubera® increasing the number of patients accepting insulin could be improved glycemic control and short- and long-term health outcomes.

## 863

**Long-term, sustained efficacy, and safety of inhaled insulin after 4 years of continuous therapy**
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**Background and aims:** Inhaled insulin (Exubera®) has shown encouraging efficacy and safety data in short-term clinical trials. This study examined whether these favorable results are maintained in the long term.
**Materials and methods:** Continued inhaled insulin therapy was offered on an open-label basis to patients with diabetes who had completed any of three 3-month, randomized, controlled clinical trials (type 1, insulin-treated type 2, or type 2 diabetes uncontrolled on oral agents [OAs]). A total of 204 patients entered the extension, with 159 electing to stay on inhaled insulin (INH) or switch to INH from comparator treatments, 89 of whom had received at least 4 years of INH therapy. In addition, a small number of patients (n = 23) receiving OAs or subcutaneous insulin was followed for 2 years.
**Results:** After 4 years, mean (±SD) $HbA_{1c}$ was 8.23 ± 1.21% in patients receiving INH compared with 8.71 ± 1.49% at the start of INH treatment. In the comparator group, the 2-year level was 7.98 ± 1.17%, compared with 8.48 ± 1.08% at baseline. INH dose increased slightly from 0.15 mg/kg after 3 months of treatment to 0.18 mg/kg after 4 years. The rate of overall hypoglycemia decreased from 2.58 episodes/subject month (first 4 weeks of INH treatment) to 1.50 after 4 years (final 6 months). Annualized changes in lung function parameters, $FEV_1$ and $DL_{CO}$ (mean ± SE) in patients receiving INH over 4 years were −0.057 ± 0.004 L/yr and −0.376 ± 0.067 mL / min/mmHg/yr, respectively. Corresponding annualized changes in non-INH patients (based on 2-year data only) were −0.071 ± 0.023 L/yr and −0.673 ± 0.423 mL/min/mmHg/yr, respectively.
**Conclusion:** Glycemic control and pulmonary function are well maintained during long-term continuous inhaled insulin (Exubera®) therapy.

## 864

**Postprandial glucose control unaffected by insulin antibodies associated with inhaled insulin**
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**Background and aims:** Insulin antibodies observed with inhaled insulin treatment do not appear to affect clinical efficacy or safety. This randomized controlled study was performed to identify any possible subtle effects on postprandial glucose disposal.
**Materials and method:** Forty-five patients with type 1 diabetes (mean age 37 years, BMI 25 kg/m², $HbA_{1c}$ 7.2%) were randomized to receive NPH-insulin BID and preprandial administration of either subcutaneous regular insulin (SC) or inhaled insulin (Exubera®; INH) for 24 weeks. After blood glucose stabilization at 130 mg/dL, 6 hourly blood glucose profiles were performed following standardized meal challenges (450 kcal, 73 g carbs, 11 g lipids) at baseline, 12 and 24 weeks. In addition, isoglycemic glucose clamp studies (target 130 mg/dL, iv insulin 0.2 mU/kg/min for 12 h) were performed the following day.
**Results:** Insulin antibody levels (mean ± SD) rose by 98 ± 140 µU/mL with INH within 24 weeks, but were stable (Δ 2 ± 5 µU/mL) with SC. Despite this difference, blood glucose profiles and glucose-infusion rate (GIR) pharmacodynamic responses were indistinguishable (Table 1). Changes in $HbA_{1c}$ (INH −0.06 ± 0.42, SC −0.08 ± 0.77%) and fasting blood glucose (−23.0 ± 86.3 vs 2.7 ± 68.1 mg/dL) were comparable, and overall hypoglycemia rate was 7.8 (INH) versus 9.4 (SC) events/subject-month.

| | INH Baseline | INH Wk 24 | SC Baseline | SC Wk 24 | 90% CI* |
|---|---|---|---|---|---|
| Maximal BG (mg/dL) | 138.4 ± 17.9 | 135.6 ± 14.1 | 142.9 ± 17.6 | 138.5 ± 11.0 | 95, 103 % |
| AUC-$BG_{0-2h}$ (mg × min/dL) | 13,395 ± 2183 | 12,855 ± 2726 | 13,188 ± 2351 | 12,977 ± 2445 | 88, 108 % |
| AUC-$GIR_{0-2h}$ (mg/kg) | 259.0 ± 124.6 | 222.2 ± 141.3 | 274.8 ± 141.2 | 279.2 ± 126.7 | 51, 111 % |
| $GIR_{max}$ (mg/kg/min) | 3.2 ± 1.2 | 2.8 ± 1.4 | 3.8 ± 1.6 | 3.5 ± 1.3 | 68, 100 % |

Mean ± SD; BG = blood glucose; *INH/SC ratio of Wk 24/Baseline ratio

**Conclusion:** Insulin antibodies associated with inhaled insulin treatment appear to have no significant impact on GIR-pharmacodynamics and postprandial blood glucose control.

## 865

**Improving quality of life in Type 2 diabetes when Exubera® is added after failure on metformin: a multicenter, international trial**
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**Background and aims:** Even though half of persons with type 2 diabetes are inadequately controlled on one oral agent 3 years after diagnosis, there is continued reluctance to start insulin until oral agent combinations fail. This is due in part to the fear, complexity and inconvenience of daily injections. To evaluate quality of life (QOL) and treatment satisfaction when initiating insulin earlier using pulmonary delivery with Exubera®, we studied 470 type 2 diabetes subjects from Europe, Africa, Asia and South America, poorly controlled on metformin monotherapy and randomized to adding either premeal Exubera®, n = 239 or glibenclamide, n = 231 for 24 weeks.
**Materials and methods:** Randomization was stratified [HbA1 c = 8–9.5% (Low) and > 9.5–12.0 % (High)] and medications were titrated to goal fasting plasma glucose of 4.4–7.8 mmol/l. Self-administered questionnaires were completed at Weeks 0 (Baseline), 10, 18, 24 and Exit, and included measures of QOL (subscales of physical, emotional and social functioning) and satisfaction with diabetes medication (subscales of advocacy, burden, convenience, efficacy, flexibility, general satisfaction, preference and side effects). Ninety percent of the eligible subjects (423/470) met the intent to treat analysis requirements for completing both a valid baseline and at least one follow-up questionnaire. In addition, 383 of those 423 subjects (91%) completed the Week 24 questionnaire.
**Results:** Patients were 57% male, and had mean age = 56 ± 9 yrs, BMI = 31.5 ± 5.1 kg/m², and HbA1 c = 9.5 ± 1.1%. Study discontinuation was 8% for Exubera® and 12% for glibenclamide, P = ns. HbA1 c strata was a treatment effect modifier for the change in HbA1 c and QOL, both P = 0.007, but not for treatment satisfaction. In the High stratum, baseline-adjusted HbA1 c decreased more for Exubera® (by 2.9 ± 0.1%) than glibenclamide (2.5 ± 0.1%), P = 0.01, but not in the Low stratum [Exubera® (by 1.5 ± 0.1%) vs. glibenclamide (1.6 ± 0.1%)], P = ns. In the High stratum, endpoint Overall QOL (scaled 100–600) was more favorable for Exubera® (468 ± 5) than glibenclamide (454 ± 5), P = 0.04, but this benefit was not evident in the Low stratum [Exubera® (457 ± 4) vs glibenclamide (467 ± 5)], P = 0.08. This modifying effect was consistent across subscales of psychological well being (P = 0.02) and vitality (P = 0.04). At endpoint the glibenclamide group reported more bed days (3.4/100 person days), an increase of 2.6/100, while Exubera® decreased from 2.2/100 to 1.1/100 (P < 0.001). Treatment

satisfaction improved comparably between groups and across strata. Improvements in HbA1c were positively correlated with improvements in general perceived health (r = 0.11, P = 0.03), general health status (r = 0.12, P = 0.014) and convenience (r = 0.15, P = 0.003).
**Conclusions:** For individuals failing metformin with HbA1c > 9.5%, adjunctive Exubera® therapy exhibited superior glycemic control, improved QOL and comparable treatment satisfaction as compared to adjunctive glibenclamide. Delays in using insulin for patients in poor control could lead to unnecessary worsening in overall well being and quality of life. When initiated earlier, alternative pulmonary delivery of insulin with Exubera® offers the good glycemic control of insulin along with improved quality of life and patient acceptance.

## 866

**Achieving target $HbA_{1c}$ in studies with inhaled insulin in Type 2 diabetes**
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**Background and aims:** The European Diabetes Policy Group (International Diabetes Federation European Region) recommends an $HbA_{1c}$ goal of ≤ 6.5% in individuals with type 2 diabetes. This analysis evaluates the effectiveness of inhaled insulin (INH, Exubera®) in achieving this goal.
**Materials and methods:** Data are from 3 open-label, randomized, parallel-group, multicenter, Phase III trials. Study 1 compared premeal INH plus a bedtime dose of Ultralente® (n = 149) with ≥ 2 daily injections of subcutaneous insulin (mixed regular / NPH insulin; n = 150) over 24 weeks in patients previously treated with insulin. Study 2 compared premeal INH (n = 105), INH plus existing oral agent therapy (n = 102), or continued oral agent therapy alone (n = 102) over 12 weeks in patients failing on combination oral agents ($HbA_{1c}$ ≥ 8%). Study 3 compared premeal INH monotherapy (n = 76) with rosiglitazone 4 mg BID (n = 69) over 12 weeks in patients failing on diet and exercise alone.
**Results:** All treatment groups had comparable $HbA_{1c}$ at baseline. Patients achieving European Diabetes Policy Group $HbA_{1c}$ treatment goals are shown in Table 1.

| Study | Treatment group | Patients achieving $HbA_{1c}$ ≤ 6.5% (%) |
|---|---|---|
| 1 | INH plus Ultralente® | 28.7 |
| | Subcutaneous insulin | 17.2 |
| 2 | INH (in combination with oral agents) | 12.2 |
| | INH monotherapy | 7.8 |
| | Continued oral therapy | 0.0 |
| 3 | INH | 28.0 |
| | Rosiglitazone | 7.5 |

INH was well tolerated (hypoglycemia and cough were the most common AEs). INH was also associated with high levels of patient satisfaction.
**Conclusion:** More patients achieved $HbA_{1c}$ ≤ 6.5% with INH compared with other regimens. INH (Exubera®) may be a valuable tool to help a wide variety of patients with type 2 diabetes reach recommended goals for glycemic control irrespective of their current therapy.

# PS 79
# Alternative insulin delivery II

## 867

**Compared intestinal uptake of insulin associated to polymere nanoparticles in diabetic and normal rats**
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**Backgrounds and aims:** Insulin, a 51 amino acid peptide, is less absorbed by the intestinal mucosa, less than 0.5 % being absorbed under physiological conditions. Previously, we have shown that the association of insulin to polymeric nanoparticles made of a non charged polymer (polycaprolactone) and a polycationic polymer (Eudragit®RS) facilitates the absorption of insulin, leading to a decrease of glycemia for a prolonged time after oral administration in diabetic rats. Since diabetes produces changes in the function and structure of the small intestine in both humans and animals, the aim of this work was to compare the uptake of insulin associated to polymeric nanoparticles in non-diabetic and diabetic rats.
**Materials and Methods:** Insulin was labelled with fluorescein isothiocyanate (FITC) as a marker. Insulin nanoparticles were prepared according to the double emulsion technique. Their size was characterized by laser light scattering. Diabetes was induced by an i.v. injection of 65 mg/kg streptozotocin in adult Wistar rats. After 4 months of diabetes induction, a 30 cm intestinal loop above the ileo-caecal junction was isolated in situ, by ligature, from the digestive continuity. The same procedure was applied to normal rats. Insulin-FITC nanoparticles (50 U/kg b.w.) were injected in the intestinal lumen. At various time intervals (5 min to 4 h) after injection, FITC levels were measured by fluorometry in the mesenteric blood, the intestinal mucosa and luminal content. Samples of intestinal mucosa were processed for fluorescence microscopy. Another experimental group, considered as control, received free FITC-insulin (50 U/kg).
**Results:** (1) The mean diameter of nanoparticles was around 350 nm. (2) The weight of the intestinal mucosa expressed per cm length was higher in diabetic rats than in normal rats (+ 74 %, $p<0.001$). (3) In both diabetic and normal rats receiving FITC-insulin nanoparticles, the concentration of FITC decreased continuously in the intestinal content from 5 min to 4 h while that in the intestinal mucosa increased from 5 min to 30 min and then decreased progressively up to 4 h. However, according to the calculated areas under the curve, the intestinal uptake of FITC-insulin was increased by 240 % ($p<0.001$) in diabetic rats and 152 % ($p<0.05$) in normal rats, by comparison with rats receiving free FITC-insulin. In mesenteric blood, FITC-insulin concentration increased progressively from 5 min to 4 h in both groups receiving FITC-insulin nanoparticles; however, FITC was undetected in the group receiving free FITC-insulin. (4) Observed by fluorescence microscopy, insulin nanoparticles appeared as fluorescent aggregates covering the upper part of the villi. Fluorescent particles appeared mainly in the follicular mucosa (Peyer's patches) since 5 min, this labelling being more intense 30 min after intra-luminal administration.
**Conclusion:** Insulin associated to polymeric nanoparticles prepared with polycaprolactone and Eudragit®RS was taken up by the intestinal mucosa. However, this uptake was more intense in diabetic rats than in non-diabetic rats. These results could be correlated with the hypertrophy of the intestinal mucosa observed in diabetic animals.

## 868

**Pharmacodynamics and pharmacokinetics of dose ranging effects of Oralin versus s.c. regular insulin in Type 1 diabetic subjects**
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**Background and aims:** The aim of the present study was to evaluate the pharmacodynamic and pharmacokinetic properties and the dose ranging effects of a buccal spray insulin formulation (Oralin) in comparison to s.c. regular insulin and to placebo spray in type 1 diabetic subjects.
**Materials and methods:** In this randomized, 5-way, crossover study, 6 subjects with type 1 diabetes received on 5 different occasions, 7 days apart: 4 doses of buccal spray ( spray placebo-10 puffs, spray insulin - 5 puffs, 10 puffs and 20 puffs, each puff contained 10 units) and one dose (0.1 U/kg) subcutaneous regular insulin, being assessed by means of euglycemic

clamp for 6 hours. All subjects received a continuous i.v. insulin infusion in the night before the clamp in order to stabilize their blood glucose levels between 90 and 100 mg/dl.
**Results:** At different doses, Oralin had an earlier onset of action (Time to early half maximal effect: 27.6 ± 12 vs 87 ± 39 min, p< 0.05), an earlier maximal effect (Tmax: 43.3 ± 18 vs 145 ± 43 min, p< 0.05) and a shorter duration of action (Time to late half maximal effect: 67.3 ± 30 vs 290.8 ± 84 min, p< 0.05) than s.c. insulin. A dose-response relationship was demonstrated by the increase in the maximal metabolic effect (GIRmax: 0.87 ± 0.54, 1.95 ± 1.32, 3.91 ± 2.51 mg/kg/min, p<0.05) and in the amount of glucose infused (GIR-$AUC_{0-120}$: 39.5 ± 34, 76.8 ± 67, 189.1 ± 163 mg/kg / 120 min, p<0.05 and GIR-$AUC_{0-120}$: 120.7 ± 106, 193 ± 185, 220 ± 189 mg/kg/ 360 min)with 5, 10, 20 puffs, respectively. For comparison, the amount of glucose infused after administration of subcutaneous insulin was: GIR-$AUC_{0-120}$ :208 ± 172 mg/kg/120 min and GIR-$AUC_{0-360}$: 890.7 ± 610 mg / kg / 360 min.
The time to maximum insulin concentration was shorter for the Oralin compared to s.c. insulin (Tmax: 26.3 ± 9 vs 142.5 ± 73 min, p< 0.05). Moreover, there was no effect of the dose on Tmax, which was similar across the dose-range studied (p: NS). The AUC of the serum insulin (Ins-$AUC_{0-120}$: 304.7 ± 277, 689.1 ± 352, 1808.8 ± 1252 µU/ml/120 min, p< 0.05) and maximum insulin levels (Cmax: 12.86 ± 8.6, 26.68 ± 14.5 and 47.6 ± 40.1 µU/ml, p< 0.05) for 5, 10 and 20 puffs respectively, proved a dose response relationship for the 3 doses of spray insulin. For subcutaneous insulin the AUC of serum insulin were Ins- $AUC_{0-120}$ : 2522.5 ± 629 µU/ml/120 min and Ins-$AUC_{0-360}$: 10268 ± 3398 µU/ml/360 min.
**Conclusion:** Oralin has a faster onset and a shorter duration of action compared to s.c. regular insulin: it is mainly absorbed and effective in the first 2 hours after administration and therefore Oralin is well suited for the management of postprandial glucose excursions.

## 869

**Linear dose-response relationship and early onset of action with inhaled human insulin using AERx® insulin Diabetes Management System in subjects with Type 1 diabetes**

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**Background and aims:** New modes of insulin treatment are being developed as alternatives to injections in order to obtain near physiological insulin levels and to increase compliance. Among those is the AERx® insulin Diabetes Management System (AERx® iDMS; NN1998) for pulmonary administration of fast acting insulin, providing flexible insulin dosing, similar to injections. This single-centre, open-labelled, five-period cross-over trial compared pharmacokinetic (PK) and pharmacodynamic (PD) dose-response relationships between pulmonary and subcutaneous (s.c.) administration of human insulin in subjects with type 1 diabetes.
**Materials and methods:** In total, 21 subjects (C-peptide ≤0.05 nmol/L; age: 39 ± 11 years (mean±SD); BMI: 23.7 ± 2.3 kg/m²; diabetes duration: 21 ± 11 years; $HbA_{1c}$: 7.5 ± 1.0%) were randomised to 5 different doses in an incomplete block design. Doses were 0.15, 0.64, 1.12 and 1.61 U/kg for inhaled insulin (dose emitted from device) and 0.03, 0.13 and 0.23 U/kg for s.c. insulin; a 10-hour isoglycaemic glucose clamp (7.2 mM) was carried out.
**Results:** The PK dose-response relationship was summarised by the area under the insulin profiles 0–10 h post dose ($AUC_{(0-10\,h)}$), the maximum insulin concentration ($C_{max}$), and time to maximum insulin concentration ($t_{max}$). The s.c. insulin doses resulted in $AUC_{(0-10\,h)}$ and $C_{max}$ values within the same range as those covered by the doses of inhaled insulin. Furthermore, $AUC_{(0-10\,h)}$ and maximum insulin concentration increased proportionally with dose after both s.c. and pulmonary insulin administration (p<0.05). Time to maximum insulin concentration increased significantly more with s.c. insulin than with inhaled insulin (p=0.003): $t_{max}$ increased significantly with higher doses after s.c. insulin, while no significant increase was observed for inhaled insulin. Overall $t_{max}$ was later after s.c. insulin than after inhaled insulin ($t_{max}$ was later for the s.c. doses 0.13 U/kg and 0.23 U/kg than for any doses of inhaled insulin, p<0.05). The PD dose-response relationship was summarised by the area under the glucose infusion rate (GIR) profiles 0–10 h post dose ($AUC_{GIR(0-10\,h)}$), the maximum glucose infusion rate ($GIR_{max}$) and onset of action (time to 10% of $AUC_{GIR(0-10\,h)}$). The lowest dose levels (s.c. insulin: 0.03 U/kg; inhaled insulin: 0.15 U/kg) resulted in very low PD responses and were disregarded. Dose-linearity was obtained for $AUC_{GIR(0-10\,h)}$ and for the maximum glucose infusion rate. No significant increase with dose was seen in onset of action for either treatment (p=0.13). However, the onset of action was statistically significantly earlier with inhaled insulin than with s.c. insulin (p<0.001). No drug related adverse events were observed during the trial.
**Conclusion:** In conclusion, dose-linearity for PK and PD parameters was obtained for both inhaled and s.c. human insulin. Onset of action occurred earlier with inhaled insulin than with s.c. insulin. Furthermore, $t_{max}$ was independent of dose for inhaled insulin, while it increased with increasing doses for s.c. insulin. Hence, a constant time from dosing to meal can be applied with inhaled human insulin using AERx® iDMS.
*Sponsored by: Novo Nordisk*

## 870

**Effect of inhaled insulin versus matched intravenous infusion on hepatic glucose balance following intraportal glucose infusion in dogs**

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**Background and aims:** Inhaled insulin (Exubera®) may be more potent than intravenous insulin due to its rapid uptake directly into the bloodstream from the alveolar bed. We tested this hypothesis using a glucose infusion model in conscious beagle dogs.
**Materials and methods:** Dogs were fitted with arterial, portal, and hepatic vein catheters 3 weeks prior to the start of the study. Fasted animals received infused insulin into the inferior vena cava (IVC; n = 10) to create an arterial PK profile identical to that of insulin delivered by inhalation (INH; n = 16). This was followed by an intraportal glucose load. Insulin was inhaled (1 mg) or infused (variable rates) at 0 min, followed at 5 min by an infusion of 0.8 µg/kg/min somatostatin to eliminate endogenous insulin (confirmed by suppressed C-peptide). Glucagon was then administered at a basal rate.
**Results:** Arterial and hepatic sinusoidal insulin rose rapidly (20 min) in the INH group to 68 ± 9 and 55 ± 7 µU/mL, falling to baseline by 185 min. Portal glucose infusion caused arterial plasma glucose to rise transiently (152 ± 9 mg/dL; 20 min), returning to baseline at 65 min where it remained for 2 hours. Net hepatic glucose uptake was minimal, while nonhepatic uptake rose to 12.5 ± 0.5 mg/kg/min by 65 min. In the IVC group, arterial and hepatic sinusoidal insulin also peaked rapidly (67 ± 2 and 55 ± 2 µU/mL; 20 min), and insulin kinetics and AUCs for both groups were identical. Arterial glucose rose rapidly (172 ± 5 mg/dL; 20 min), and transiently fell to 131 ± 13 mg/dL at 65 min before returning to 170 ± 5 mg/dL by 125 min. Plasma glucose excursions were much larger in the IVC group (10,345 ± 2362 mg/dL/360 min) compared to the INH group (2533 ± 2309 mg/dL/360 min). Hepatic glucose uptake was also greater (-4.7 ± 1.4 mg/kg/min; 65 min) and nonhepatic uptake markedly less (8.3 ± 0.5 mg/kg/min; 20 min).
**Conclusion:** Inhaled insulin (Exubera®) caused significantly greater peripheral glucose uptake and hence better overall glycemic control following a simulated oral glucose load than IVC insulin, despite matched plasma insulin levels.

## 871

**Complete mapping of the receptor-binding surface of the insulin molecule by site-directed mutagenesis: implications for analogues design**

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**Background and aims:** Recent data suggest that two separate binding surfaces on the insulin molecule are involved in high affinity binding by crosslinking the two alpha-subunits of the insulin receptor, thereby triggering intracellular signalling. While one binding surface (overlapping with the dimer-forming surface) has been identified many years ago by structure-function studies of naturally occurring or modified insulins and more recently by alanine-scanning mutagenesis, the putative second surface has not been precisely mapped. Studies of insulins mutated at A13 and B17 (in the hexamer-forming surface) had shown anomalous binding behaviour suggesting that these residues may also be involved in receptor binding. We now report the complete mapping of the bioactive surface of insulin by systematic site-directed mutagenesis using the cell-bound high affinity human insulin receptor.

**Materials and methods:** Insulin analogues were expressed in yeast, bearing single or double alanine mutations in the dimer- and hexamer-forming surfaces of the molecule, as well as substitutions found in hystricomorph and hagfish insulins which display anomalous binding behaviour similar to A13 and B17-mutated insulins. Their binding affinity for the human receptor on IM-9 cells was measured by radioligand competition assays.
**Results:** Alanine substitutions that caused more than 2-fold decreases in binding affinity in binding to the IM-9 human receptor mapped either to the "classical" binding surface (residues A1, A2, A3, A5, A8, A19, A21, B12, B16 and B23-26), or to a novel surface that overlaps with the hexamer-forming surface of insulin (A12, A13, A17, B10, B13, B17). In addition, substitution of B14 Ala to Ser (only adding an -OH group) caused an 8-fold decrease in affinity, suggesting that B14 is close to the binding interface. Substitutions found in hystricomorph or hagfish insulins in this novel surface (at A13, A17, B17) as well as in the dimer-forming surface (B26) explain the low affinity of these insulins, compensated by substitutions with a positive effect at A8, A18, B10 and B20 (the latter not previously thought to influence insulin receptor binding). Double alanine substitutions showed an additive effect.
**Conclusion:** These data support the bivalent crosslinking receptor binding model, and portray a more complete picture of the insulin-receptor binding interface, which is more extensive than previously thought, and have profound implications for the design of clinically useful insulin analogues.

## 872

**Insulin bio-effect is limited by speed of absorption and elimination: similarities between an inhaled insulin formulation that mimics first-phase kinetics and i.v. insulin**

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**Background:** In normal humans, peri-prandial insulin (INS) release is biphasic, with a „reflex-like" first-phase of short duration followed by a broader second-phase release related to nutrient absorption. The loss of the first-phase response (FFR) is an early occurrence in progression to type 2 diabetes. The kinetics of absorption of current INS preparations has limited the ability to emulate the FFR with practical therapy. We compared the pharmacokinetics and pharmacodynamics of INS administered via intravenous (IV) and subcutaneous (SC) routes with those of Technosphere®/Insulin (TI), a new inhaled INS which has a fast onset of action and relatively high bioavailability (BA).
**Methods:** In study #1, 5 healthy volunteers (HV) were evaluated on three separate study days during which they received regular human insulin (rHI), either as 5 IU IV, as 10 IU SC or as 100 IU TI. In study #2, 12 HV received rHI, either as 15 IU SC, or as 25 IU TI, 50 IU TI or 100 IU TI. In both studies, euglycemic clamp maintained glucose levels at 5 mmol/L. Endogenous INS production was suppressed with INS infusion at 0.15 mU/kg/min.

Results: Study 1

| Insulin | 5 IU IV | SD | 10 IU SC | SD | 100 IU TI | SD |
|---|---|---|---|---|---|---|
| $Tmax_{INS}$ (min) | 5 | 4 | 126 | 65 | 13 | 5 |
| $T_{1/2INS}$ (min) | 10 | 8 | 239 | 78 | 36 | 5 |
| $Cmax_{INS}$ (mIU/L) | 615 | 214 | 34 | 14 | 371 | 65 |
| $AUC_{INS}$ (0–360) (mIU/L) | 5746 | 1440 | 6651 | 2322 | 16982 | 2299 |
| Glucose Infusion | | | | | | |
| GIRmax (mg/kg/min) | 7.1 | 2.2 | 6.2 | 1.5 | 8.3 | 3.0 |
| AUC (0–360) (mg/kg) | 349.0 | 149.1 | 1000.1 | 246.8 | 985.2 | 488.3 |

**Results: Study 1** - The initial absorption rate of TI to $T_{max}$ was as fast as that of IV, but INS levels were elevated longer with TI. rHI administered SC was absorbed more slowly, with 65.3% recovered in the first 180 min, compared to 97% for IV and 93% for TI. The required glucose infusion rate (GIR) increased rapidly and with similar slope after both IV and TI but more slowly after SC. The ratio of $AUC_{GIR}(0–360)/AUC_{INS}$ (0–360) was 0.058 and 0.061 respectively for TI and IV and 0.150 for SC.

Results: Study 2

| Insulin | 10 IU s.c. | SD | 25 IU TI | SD | 50 IU TI | SD | 100 IU TI | SD |
|---|---|---|---|---|---|---|---|---|
| $C_{INS}$max (mIU/l) | 25 | 11 | 55 | 37 | 110 | 41 | 189 | 98 |
| $AUC_{INS}$ (0–360) (mIU/l) | 5294 | 2028 | 4156 | 3138 | 8196 | 6396 | 12493 | 6430 |
| Glucose Infusion | | | | | | | | |
| GIRmax (mg/kg/min) | 6.3 | 2 | 5.2 | 1.1 | 6.2 | 2 | 7.0 | 2.5 |
| $AUC_{GIR}$(0–360) (mg/kg) | 1058.3 | 338 | 641.7 | 252 | 721.0 | 239 | 862.1 | 402 |
| $AUC_{GIR}$ (0–360)/ $AUC_{INS}$ (0–360) | 0.200 | | 0.155 | | 0.089 | | 0.069 | |

**Results: Study 2** - Both $GIR_{max}$ and total GLU disposal increased with increasing TI dose. However, the increment in $GIR_{max}$ and total GLU disposal was not proportional to the dose or BA. $AUC_{GIR}(0–360)/AUC_{INS}$ (0–360) relative to SC, was 77%, 44% and 35% for 25 IU, 50 IU and 100 IU TI respectively.
**Conclusion:** The effect of TI on $GIR_{max}$ relative to dose decreased with increasing doses and approached an „$E_{max}$" as has been previously shown with incremental acute IV bolus. Total GLU disposal relative to BA was similar for TI and IV, while both were only 35–40% of that seen with SC. The ability of TI to mimic FFR and approximate the $E_{max}$ relationship shown with IV bolus provides opportunity to explore this dynamic in ambulatory diabetes management.

# PS 80

# Evaluation of blood glucose monitoring systems

## 873

**Accuracy evaluation of TrueTrack Smart System™, a new blood glucose measurement device suitable for alternate site testing**

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**Background and aims:** TrueTrack Smart System™ is a new developed blood glucose self-monitoring device that allows for measurements at the fingertip and at the forearm (Alternate Site Testing). The goal of this multi center clinical trial was to evaluate the accuracy of this device in comparison to commercially available state-of-the-art blood glucose meters. The study was performed according to ICH/GCP-guidelines and received approval from ethical review board.
**Materials and methods:** Blood glucose measurements were performed both at the fingertip and the forearm by diabetes healthcare professionals at 3 study centers. Data sets were collected from 102 diabetic patients (34 women, 68 men, age: 55.5 ± 12.9 years; BMI: 29.2 ± 5.4 kg/m$^2$; mean duration of diabetes in 23 type 1 diabetic patients: 17.7 ± 10.5 years, and in 79 type 2 diabetic patients: 10.1 ± 7.5 years).Up to 4 test series per patient were performed during 2 test visits. The comparator devices were FreeStyle™, Ascensia® Contour™, OneTouch® Ultra, and Accu-Chek® Comfort (fingertip only). During each test series investigational and comparator devices were used in randomized order testing at the fingertip and the forearm. Each blood glucose self-monitoring system was applied with 3 lots of test strips and 9 sensors in randomized order. A laboratory reference measurement was performed at the beginning and the end of each test series by means of a glucoseoxidase reference method (Super GL). For each test series the mean of both laboratory readings was used as reference value for analysis.
**Results:** All devices correlated well with the laboratory reference method: TrueTrack Smart System (fingertip: r=0.946/forearm: r=0.929), Freestyle (0.961/0.933), Ascensia Contour (0.934/0.895), OneTouch Ultra (0.943/0.898), and Accu-Chek Comfort (0.949/–). Linear regression analysis revealed as follows: TrueTrack Smart System (fingertip: slope=1.006, intercept=10.7 mg/dL/forearm: slope=0.913, intercept=27.3 mg/dL), FreeStyle (1.042, –3.6/0.933, 5.6), Ascensia Contour (1.045, 0.8/0.923, 8.4), OneTouch Ultra (1.045, 2.7/0.931, 15.4), and Accu-Chek Comfort (1.062, 3.4/–). Standard deviation of the mean at linear regression analysis was observed as follows: TrueTrack Smart System (fingertip: STD ERR=16.237 mg/dL/forearm: STD ERR=17.122 mg/dL), FreeStyle (13.937/16.743), Ascensia Contour (18.476/21.388), OneTouch Ultra (17.146/21.180), and Accu-Chek Comfort (16.344/–). A high quality standard of all study devices was shown at the Error-Grid-Analysis according to Parkes. All measurements were within the clinical acceptable zones A and B.
**Conclusion:** In conclusion, for both fingertip testing and AST at the forearm TrueTrack Smart System™ has shown comparable accuracy and reliability to competitive state-of-the-art blood glucose monitoring systems.

## 874

**Accuracy and ease of use of a new blood glucose monitoring system and examination of the sample glucose distribution**

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**Background and aims:** To evaluate the clinical accuracy and ease of use of the new Precision Xceed™/Optium Xceed™ (MediSense, Abbott Laboratories) blood glucose monitoring system (BGMS) using ISO15197 criteria as a benchmark for performance. The specifications of this new system include a 10second test time and a sample volume requirement of ≥1.5 µL. The sample distribution of blood glucose measured is also examined and compared with the ISO recommendations for an accuracy study. ISO recommends a distribution of samples across seven levels, but only allows fresh unaltered samples to be tested at glucose concentrations of 2.8 mmol/L to 22.2 mmol/L. At higher or lower glucose concentrations, artificially produced samples maybe included.
**Material and methods:** During four weeks, 124 adults with diabetes (22% Type 1) attending an out-patient clinic were recruited to perform self-testing on the BGMS using the instructions for use, and donate a fresh capillary sample for reference testing on the Yellow Springs Instruments (YSI) whole blood glucose analyser. After testing the lay users rated the BGMS for six aspects of ease of use.
**Results:** 100% of BGMS results fell within ± 0.83 mmol/L and ± 20% of the reference at levels <4.2 mmol/L and ≥4.2 mmol/L respectively. Parkes Error Grid analysis found 99.6 and 0.4% of results in zones A and B respectively. Regression analysis yielded slope=0.96, intercept=0.07 mmol/L, r=0.99, $S_{y,x}$=0.38. Paired replicate coefficient of variation of BGMS results was 2.7%. On a satisfaction scale of 1 (lowest) to 6 (highest) for aspects of ease of use the mean score was 5.7. Users liked the small size, speed, and simplicity of the BGMS.
The distribution of the samples covered a reference range of 3.8–25.0 mmol/L. ISO recommends a minimum of 100 samples with 15% in level 2, range 2.8–4.4 mmol/L. For this population we obtained 4 samples in this range and so estimate an average of 370 samples would need to be screened to obtain 15 samples in this range, though this could vary statistically from 230 to 540.
**Conclusions:** The Precision Xceed™/Optium Xceed™ BGMS was liked by patients and was easy to use. The system demonstrated excellent accuracy and precision over a wide range of glucose values. The ISO15197 sample distribution requirements in the lower range (2.8–4.4 mmol/L) are difficult to achieve. In order to conduct a study to exactly meet the specified sample distribution extended screening, a multi-centre study or a different donor population may be required.
*Supported by Abbott Laboratories, MediSense*

## 875

**Accuracy of real-time glucose values using continuous glucose monitoring: the Guardian™ II continuous glucose monitoring system**

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**Background:** The Guardian™ II Continuous Glucose Monitoring System (Medtronic MiniMed) adds a real-time glucose display to the hypo- and hyperglycemia alert function of the Guardian™ System. We evaluated the accuracy of the real-time glucose values of the Guardian II by comparing subcutaneous sensor glucose to venous blood samples collected every 30-minutes using the YSI 2300 Stat Plus Glucose Analyzer.
**Methods and Results:** Sixteen subjects wore two Guardian devices simultaneously for 72 hours in an in-patient setting. Guardian devices were calibrated using plasma glucose values obtained from the YSI. The median (interquartile range) age of the 13 subjects (8 male) contributing demographic data was 42 years (30.5 to 50 years), duration of diabetes was 22 years (6 to 29.5 years), and HbA1 c was 8.1% (7.6 to 8.9%). A total of 38 sensors resulted in 86 days of device experience with median sensor life of 61 hours (44 to 71 hours).

| | Calibrations per day (N) | Pairs (N) | MAD (N) | Correlation | Clarke Error Grid Zones A+B |
|---|---|---|---|---|---|
| **Accuracy** | | | | | |
| Sensor-YSI Pairs | 5.1 | 4,037 | 17.95 % | 0.82 | 95.9% |
| Sensor-YSI Pairs | 3.9 | 3,432 | 18.54 % | 0.82 | 96.0% |
| **Precision** | | | | | |
| Sensor-Sensor Pairs | 5.1 | 11,402 | 20.7 % | 0.79 | 96.2% |

**Conclusions:** The Guardian II accurately provides real-time sensor glucose values to the user and these values are reproducible between sensors. These results suggest that once commercially available, the Guardian II will be valuable in managing glycemic control. Additional studies will determine whether the Guardian II can improve metabolic outcomes.

## 876

**Evaluation of seven blood glucose monitoring systems of the accuracy for the patients use**

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**Background and aims:** In order to investigate the cause of inaccurate test results on blood glucose monitoring systems, the effects of insufficient samples and interfering materials were evaluated using seven blood glucose monitoring systems.
**Materials and methods:** The seven blood glucose monitoring systems were used in this study. The precision test with sufficient blood samples (3 different concentration of glucose with replicate 10 × 2 instruments on each systems) were performed then the effects of applying insufficient blood on each system were evaluated by decreasing the applied blood volume gradually. All test results were compared to the mean glucose value obtained with sufficient blood volume on each system. In addition, the effects of other sugars (maltose and galactose) and reducing substances (ascorbic acid, uric acid, gentisic acid and combination of three materials) were investigated by adding them to the blood glucose sample and glucose value with those materials are compared with non-added blood glucose samples.
**Results:** The coefficient of variance (%) with sufficient blood were, Aventice GlucoCard: 1.7~5.1%, Sanwa Glutest Neo: 1.2~2.6%, Abbott Precision Xtra: 1.1~2.8%, J&J One Touch Ultra: 1.4~2.5%, Roche AccuCheck Compact: 1.2~3.1%, Terumo MediSafe-Mini: 3.1~8.3%, Bayer Breeze: 1.6~8.8%, respectively. By decreasing the applying blood volume, the GlocoCard showed abnormally lower results (6~48% values comparing the results of sufficient blood volume). Four systems (Glutest Neo, One Touch Ultra, AccuCheck Compact and Breeze) displayed error message and test strip showed be wasted. The Precision Xtra and the Medisafe-Mini did not start and the Precision Xtra was possible to re-apply additional blood with-in 30 seconds. Regarding the effects of other sugars, Glutest Neo and AccuCheck Compact showed remarkably higher results (252~261 mg/dl higher by adding 360 mg/dl of maltose, 166~177 mg/dl higher by adding 180 mg/dl of galactose) and no effects were observed on other five systems. Regarding the reducing substances, the Midisafe-Mini showed decreased glucose value and four systems (GlucoCard, Glutest Neo, One Touch Ultra, Breeze) showed increased glucose value. Those effects were accumulated when the materials were combined. The effects of reducing materials on Precision Xtra and AccuCheck Compact were lower than other systems.
**Conclusion:** The effects of insufficient samples and interfering materials were variable on the systems.In order to keep the accuracy for patient use, medical staffs should consider to 1.Understand features and performance of the each blood glucose monitoring system, 2.Instruct to the patient to use enough blood and 3.Monitor the medical and endogenous data of the patients. Within the seven monitoring systems, Abbott Precision Xtra showed the best feature and performance to avoid inaccurate test results on the self-monitoring of the patients.

## 877

**Evaluation of the accuracy of the continuous interstitial glucose monitoring device GlucoDay® under sequential stepped eu- hypo- and -hyperglycemia in non-diabetic subjects**

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**Background and aims:** To evaluate the accuracy of the continuous interstitial glucose monitoring device GlucoDay® (A. Menarini Diagnostics, Italy) and its relationship with arterialized-venous blood glucose (AVBG) in non-diabetic subjects.
**Materials and methods:** we studied 9 subjects under sequential steps of euglycemia (30 min, 85 mg/dl), hypoglycemia (30 min, 50 mg/dl), and hyperglycemia (15 min, 150 mg/dl) (hyperinsulinemic glucose clamp technique; plasma insulin 80 ± 16 µU/ml, mean±SD) after 166 ± 47 min of calibration.
**Results:** The overall study duration was 180 min, AVBG measurements were taken at 3 or 6 min intervals and were compared with glucose estimates provided by GlucoDay®. We analyzed 275 sets of data that paired AVBG readings and GlucoDay® values and found the coefficient of correlation (R) being 0.69. The ADA precision criteria to within ± 10% were satisfied in 34% of cases. Eighty-four per cent of paired samples were in the clinically acceptable A and B zones of Clarke error grid analysis. The bias was 14 ± 40 mg/dl. Interestingly, we found an inverse correlation (R=–0.74) between the accuracy of the GlucoDay® and the variation of the current signal during baseline euglycemia. In fact, statistical analysis of data of those patients (N=6) who exhibited a lower fluctuation in the current signal (coefficient of variation <10%) revealed greater accuracy (ADA precision criteria 41%) and had more paired values falling in the acceptable A and B zones (93% of 184 paired data). R was 0.9 and the bias was 0.007 ± 16 mg/dl, respectivel.
**Conclusion:** We conclude that the continuous interstitial glucose monitoring device GlucoDay® is closely correlated to AVBG during sequential steps of eu-, hypo- and hyperglycemia in non-diabetic subjects. However, this result is strictly dependent on the stability of the current signal prior to calibration in euglycemia. Stability of current signal is crucial to obtain accurate measurement of interstitial glucose concentration

## 878

**The Medtronic Minimed Continuous Glucose Monitoring System for patient use: real-time sensor glucose values and trend information**

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**Background:** Continuous glucose monitoring should improve glycemia and decrease complications in patients with diabetes. The sensor-transmitter from the Medtronic MiniMed Guardian™ II Continuous Glucose Monitoring System collects sensor signals and periodically transmits glucose data using a radio signal to the Guardian II receiver that displays values in real-time. As a first step toward combining the sensor with the insulin pump, the pump platform was modified to receive sensor signals, but not pump insulin. The Paradigm 522 pump receives radio signals from the Guardian II transmitter every 5 minutes, interprets the signals, displays values and trend graphs, and stores up to 30 days of data. Patients wearing the device continue to self-monitor blood glucose (BG) using a BG meter and deliver insulin using their existing insulin pump. BG meter values are automatically transferred to the 522 receiver and stored in its memory as a calibration point. This study was designed to evaluate the accuracy of the Paradigm 522 receiver, while providing real-time sensor glucose values to patients and clinicians.
**Methods and results:** Ten children with a median (range) age of 14.5 years (10 to 18 years), type 1 diabetes, and on CSII therapy inserted and wore 7 sensors over a 30-day period. Accuracy endpoints were paired sensor and BG values obtained from the 522 receiver and the BG meter. Five subjects wore sensors for 30 days while an additional 5 subjects wore sensors for 15 days and are currently completing the study. There were a total of 133.9 days of device experience. The mean (median) absolute difference between the 949 sensor-meter pairs was 19.6% (14.3%), correlation was 0.86, and 95.3% of the sensor-meter pairs fell within Clarke error grid zones A and B.
**Conclusions:** The Paradigm 522 receiver provided real-time sensor glucose values that correlated well with discrete BG readings and graphical trend information. The Paradigm 522 System holds the potential to enable patients with diabetes to improve glycemia and long-term diabetes outcomes.

## 879

**Determination of instrument and lot imprecision for six different blood glucose analyzers used in point-of-care testing**

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**Background and aims:** Instruments for self-monitoring of glucose (SMBG) are increasingly used by diabetic patients. In addition, these instruments are used frequently now for Point-of-Care Testing (POCT) in hospitals. The results of many evaluation studies have already been published including inter- and intra - assay imprecision, linearity, comparison with laboratory methods and different sources of interferences. Only a few studies with limited numbers on differences between individual instruments and test strip lot to lot differences have been published so far. With the increasing number of POCT instruments in hospitals that easily sum up to one hundred in large community hospitals these differences have to be considered in the total quality management of POCT.
**Materials and methods:** Instrument and test strip/microcuvette lot imprecision were determined for HemoCue Glucose 201+ with microcuvettes (Hemocue AB), Ascensia Elite XL with Ascensia Elite XL sensor (Bayer Vital GmbH), Ascensia Contour with Ascensia Microfill (Bayer Vital GmbH), Medisense Precision Xtra with Precision Xtra Plus (Abbott Laboratories), OneTouch Ultra (Lifescan) and Accu-Check Sensor with Accu-Check Sen-

sor Comfort (Roche). For the determination of instrument imprecision 10 instruments of each analyzer were used and glucose measurement was done at the same time for each instrument of a kind. Whole blood samples (EDTA without NaF) from patients were used to ensure equal quality of blood samples for each instrument. Test strip lot to lot imprecision was determined with whole blood samples (EDTA without NaF) from patients using three different test strip/microcuvette lots on one instrument.
**Results:** A total of 6950 measurements was done for the instrument imprecision determination with glucose concentrations from 30 to 385 mg/dl. Mean and relative standard deviation (RSD) was calculated for every patient sample measured on the ten instruments. For the different instruments the mean, minimum and maximum RSDs were calculated. The mean RSDs (instrument imprecision) were 2.1% (Elite), 2.5% (Ultra), 2.8% (Precision), 3.3% (HemoCue), 3.4% (Accu-Check) and 3.8% (Contour) with maximum RSDs of 5.5% (Elite), 11.1% (Ultra), 11.3% (Precision), 10.9% (HemoCue), 13.0% (Accu-Check) and 10.4% (Contour). A total of 1320 measurements was done for the lot to lot imprecision determination with glucose concentrations from 30 to 387 mg/dl. Mean and relative standard deviation (RSD) was calculated for every patient sample measured on one instrument with three different lots. For the instruments the mean, minimum and maximum RSDs were calculated. The mean RSDs (lot imprecision) were 1.8% (HemoCue), 2.9% (Contour), 3.0% (Accu-Check), 3.3% (Elite) and 3.9% (Precision) with maximum RSDs of 7.8% (HemoCue), 11.8% (Contour), 12.4% (Accu-Check), 11.6% (Elite) and 16.9% (Precision).
**Conclusion:** The determination of instrument to instrument imprecision show similar results for all analyzers tested with a slightly better performance of Ascensia Elite XL. The determination of lot to lot imprecision revealed higher RSDs compared to instrument to instrument imprecision with a better performance of HemoCue Glucose 201+ analyzer. In the future these differences have to be considered in the total quality management of POCT next to accuracy, inter- and intra - assay imprecision and linearity of an individual analyzer.

## 880

### On-line glucose prediction from continuous monitoring data and prevention of hyper/hypo-glycaemic events

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**Background and aims:** Continuous glucose monitoring (CGM) by minimally-invasive sensors can enable the design of appropriate changes in diabetes management in order to achieve the goal of optimal metabolic control. At the same time, a portable CGM system could be used as a tool to generate on line alarms in presence of hypoglycemic/hyperglycemic events. The aim of the study was to assess off-line the possibility to predict with some advance glucose levels by exploiting their recent history monitored for 48 h every 3 min by the GlucoDay system in 13 type 1 diabetic patients (7 males, 6 females, mean age 43 ± 4 years, duration of the disease 17 ± 3 years, HbA1 c 8 ± 0.3 %).
**Materials and methods:** A simple prediction strategy, potentially usable on-line and based on 1st order polynomial modeling of the data, was evaluated by considering two different prediction horizons (PH), i.e. 30 and 45 min. At each sampling time a polynomial model of order 1 is first fitted by weighted linear least squares against the past data and then used to predict the glucose level after a given PH. In model fitting, the weight m^k is assigned to the sample relative to k instants before the actual sampling time, with m being a parameter which behaves like a forgetting factor. In order to assess the method, we have first evaluated how well, in terms of time location, the major peaks (on average 4.38 per subject) and nadirs (on average 4.15 per subject), preliminarily identified on the original time-series, can be detected from the predicted time-series.
**Results:** When PH=30, the average delays (SD) of peaks and nadirs are respectively 10.44 (12.04) and 11.93 (11.82) (m=0.2), 12.99 (12.70) and 11.66 (12.42) (m=0.5) and 21.01 (10.67) and 18.83 (10.84) (m=0.8). When PH=45, the average delays (SD) are 16.10 (19.57) and 17.62 (17.97) (m=0.2), 23.83 (13.87) and 19.99 (18.27) (m=0.5), 30.74 (14.38) and 25.24 (18.47) (m=0.8). Then, we have quantified the delay also during negative and positive trends. In particular, we have measured the time at which some thresholds (when the trend is negative placed at the 75% of the peak-to-nadir distance, when the trend is positive placed at the 75% of the nadir-to-peak distance) are crossed in the original and in predicted curve. When PH=30, the average delays (SD) during positive trends (on average 3.86 per subject) and negative trends (on average 4.15 per subject) are −4.71 (14.06) and −0.40 (13.53) (m=0.2), −1.23 (11.38) and 2.43 (12.47) (m=0.5), 8.04 (13.84) and 5.70 (11.79) (m=0.8). When PH=45, the same quantities are −0.35 (17.11) and 3.78 (16.12) (m=0.2), 3.53 (16.35) and 5.80 (16.55) (m=0.5), and 10.92 (20.34) and 15.84 (18.25) (m=0.8). Remarkably, peaks and nadirs and threshold crossings can be detected with a delay which is significantly lower than PH. The smaller is m, the smaller is the delay but the greater its variability with the inherent possibility of generating false alarms
**Conclusion:** In conclusion these results encourage further developments of prediction strategies suggesting that hypoglycemic/hyperglycemic states could be predictable in advance on the basis of the past recent glucose concentration data provided by GCM systems.

# PS 81

# Monitoring and acute glycaemic emergencies

## 881

**Detection of asymptomatic ketonemia and its relation with hyperglycaemia in Type 1 diabetic patients**

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**Background and aims:** Increased levels of blood ketones are clinically relevant in type 1 diabetes and pregnancy. Although it is advisable to test ketones when glycaemia is > 250 mg/dl (13.9 mmol/l), the relation between blood ketones and glycaemia levels is not well established yet. The recent introduction of a technique that measures the concentration of β-hydroxybutirate (β-OHB) in capillary blood, allows patients to easily control ketosis when it happens without evident symptomatology, and also helps to correct quickly any possible subjacent metabolic alteration. The present study was conducted with the aim to assess the prevalence of asymptomatic ketosis in type 1 diabetic patients in a population having casual hyperglycaemia.
**Materials and methods:** To assess prevalence, an one-month observation period was fixed in all 7 Spanish Endocrinology units (2 in Madrid, Las Palmas, Lleida, Seville, Oviedo and Barcelona). Glycaemia from 562 type 1 diabetic patients was recorded in this period. For patients with > 250 mg/dl (13.9 mmol/l) urine was also measured (Ketodiastix®, Bayer) as well as blood ketones (Optium®, Abbott MediSense) and further study variables were recorded. Ketosis was considered when blood ketones were ≥ 0.5 mmol/l. For statistical analysis, SPSS 11.5 was used.
**Results:** Prevalence of casual hyperglycaemia was 27.58 % (present in 155 patients), and prevalence of asymptomatic ketosis was 2.31 % (present in 13 patients). This means that 8.39 % of patients who have casual hyperglycaemia present at the same time asymptomatic ketosis. Regarding the different levels of blood ketones, 110 out of 155 (71 %) had blood ketones between 0.0 and 0.1; 32 out of 155 (20.6 %) had blood ketones between 0.2 and 0.4; and 13 out of 155 (8.4 %) had blood ketones ≥ 0.5 mmol/l. Surprisingly, the mean of glycaemia in these subgroups did not differ (not statistically significant intergroup differences). In 91.6 % of hyperglycaemias and 69 % of ketonemias, patients were in non-fasting state.
**Conclusion:** 1. The presence of ketosis even bellow the levels considered as pathologic, detected by β-OHB levels together with hyperglycaemia, must be taken into account for a proper clinical and therapeutic practice. 2. The relationship between glycaemia and ketonemia is not always constant. 3. Asymptomatic ketosis was observed in the type 1 diabetes population, and therefore metabolic control of the patients with new technologies and subsequent revision of insulin treatment is advisable.
*Supported by: Abbott Científica SA*

## 882

**Diabetic ketoacidosis: errors and pitfalls of management on primary care level**

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**Background and aims:** To analyze the typical errors and pitfalls of diabetic ketoacidosis (DKA) management on primary care level to improve the outcomes of DKA, which is the main death reason during the first decade of type 1 diabetes.
**Materials and methods:** The analysis of 120 cases of DKA emergencies in type 1 diabetes patients (age 12–40 years) admitted to local hospitals.
**Results:** The analysis of the situations developed during first hours or days of DKA management on primary care level has revealed errors, pitfalls, difficulties and enabled us to come to following conclusions and to suggest some recommendations, which are worth emphasizing on the background of known from literature information. 1. The main marker of the severity of type 1 diabetes decompensation is a degree of acidosis, which often persists many hours with hyperglycemia 10–15 mmol/l (euglycemic DKA), especially in young girls and pregnant woman. Dehydratation increases rapidly after vomiting (one of DKA sign) begins. 2. Overbreathing (and Kussmaul respiration) is the main early, specific DKA sign which often is overlooked or considered as the sign of heart or respiratory insufficiency; every unclear hyperpnea is to be considered as sign of possible metabolic acidosis (DKA first of all). 3. The acidosis severity depends predominantly on increase of beta-oxibutyrate concentration and its measuring is very helpful. 4. Carbohydrate hunger due to vomiting, dietary restriction (and even mild hypoglycernia) often precipitate or maintain DKA. Vicious circle develops in severe cases: vomiting -DKA - persisted vomiting - more severe DKA and dehydratation ... .Such situation is not rare during perioperative period or renal insufficiency in diabetic patients. Glucose-insulin-potassium (GIK) infusion (after glycemia is lower than 15 mmol/1) proved to be effective to break such circle. The rate of glucose infusion is about 8 g/hour; vitamin Bl must be added. In euglycemic DKA the management may be started with GIK infusion. 5. Rehydratation : crystalloid fluids must be used during first three hours, than - colloids if it is necessary (avoiding dextrans). Overenthusiastic infusion of hypotonic fluids (in situation without hypematriemia) was the main reason for fatal brain edema in two our cases due to disequilibrium syndrome. 6. Intravenous bolus insulin regime is dangerous: DKA persists (fatal outcome in 4 cases). 7.Potassium deficit must be replaced as soon as possible.
**Conclusion:** Primary care medicine is the first and often the last stage DKA management. Therefore, it is necessary to consult medical staffs by specialists continuously and to teach guidelines of DKA management. Moreover, patients must be warned about possibility of DKA and informed how to avoid, recognize in time and stop (if vomiting absent) DKA development at very beginning.

## 883

**Diabetic ketoacidosis, hyperglycaemic hyperosmolar non-ketotic states. Are we getting it right in the 21st century?**

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**Introduction:** Hyperglycaemic diabetic emergencies are increasing a less frequent occurrence as medical admissions in the UK. This is in part secondary to the establishment of Diabetes centres, where during office hours early intervention and advice can be administered preventing Diabetic ketoacidosis (DKA) and Hyperosmolar Non-Ketotic Coma (HONK). The traditional mortality quoted for DKA and HONK are <5 % and 15% respectively, with modern fluid management the mortality for DKA is probably approximately 2 %.
**Methods:** The Central Middlesex Hospital has prospectively collected data on all admissions with DKA, HONK and Hyperglycaemic Non Ketotic states from 1999. The data presented covers the period 01/01/2000 to 21/01/2004. DKA is defined as plasma glucose greater than 15 mmol/L with an arterial pH < 7.30 and ketonuria. HONK was defined as an osmolarity ≥320 mosmol/L with an arterial ph ≥ 7.30. The remainder were classified as hyperglycaemia requiring medical admission (HRA). Adherence to established protocols for the management of DKA and HONK was also audited.
**Results:** There were 159 admissions (from 140 individuals, 1 individual had 5 separate admissions another 4 separate admissions and 5 individuals who had 2 admissions). There was one admission with lactic acidosis.

Table 1 summarizes the data for the 3 groups

| | All | DKA | HONK | HRA |
|---|---|---|---|---|
| N (number of admissions) | 159 | 46 | 57 | 55 |
| Sex (F/M) | 57/101 | 20/26 | 15/42 | 22/33 |
| Age (yrs) | 47.4 ± 18.8 | 38.4 ± 16.6 | 55 ± 17.5 | 46.4 ± 19.0 |
| Ethnicity (%) | | | | |
| African/Caribbean | 41 | 28 | 53 | 38 |
| Indian Subcontinent | 19 | 17 | 21 | 18 |
| Europid | 32 | 48 | 18 | 35 |
| Middle-east | 3 | 2 | 5 | 0 |
| Other Asian | 4 | 4 | 2 | 5 |
| pH | 7.37 ± 0.11 | 7.29 ± 0.11 | 7.39 ± 0.09 | 7.44 ± 0.06 |
| Glucose (mmol/L) | 37.1 ± 18.8 | 31.2 ± 9.8 | 51.0 ± 21.2 | 25.5 ± 8.7 |
| Deaths | 5 | 1 | 4 | 0 |

**Discussion:** There where 5 deaths over the 3 year period a mortality rate of 3.1%, mortality in the DKA group was 2.2% and in the HONK group 7%. Identified risk factors for mortality were previous CVA, hypertension, treatment with a calcium channel antagonist, residency in a nursing home,

male sex and an African Caribbean ethnic origin and admission under a diabetologist. With modern fluid management the mortality from DKA and HONK is lower than previous reported. HONK retains a high mortality especially in vulnerable individuals. The increased mortality in patients admitted under the diabetologists may reflect local practice, which encourages early notification of diabetic emergencies to the diabetes team.

## 884

**Ketoacidosis in Type 2 diabetes may occur with hypergycaemia and euglycaemia**

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**Background:** We describe a series of cases illustrating the spectrum of glucose profile (raised & normal) in ketoacidosis associated with type 2 & gestational diabetes.
**Method:**
**1:** Male aged 19 years had abdominal pain and vomiting. BMI 33, smelt ketotic. Lab tests : glucose 66 mmol/l, CRP 174 mg/l, white cell count (WCC) 34100/dl. Blood gases: $H^+$ 77 mmol/l (normal 35–44 mmol/l), bicarbonate 3 mmol/l. Urine ketones + + + +. Liver function tests (LFT) normal & serum amylase 765 mmol/l. Diagnosed diabetic ketoacidosis and treated with intravenous (IV) insulin, fluids & antibiotics. CT scan of abdomen: no pancreatic pathology. Blood cultures, viral studies and atypical serology negative. Random non fasting unstimulated C peptide 1817 pmol/l (normal 140–1390) & insulin 55.8 mU/L (normal 0–16). Antibodies to GAD and beta islet cells were negative. Total cholesterol 4.6 mmol/L. Insulin stopped 6 months later. After 14 months, $HbA_1C$ 6% on metformin alone .
**2 :** Female aged 56 years had right upper quadrant abdominal pain & vomiting. BMI 32, smelt ketotic, temperatue 38°C, pulse 100/minute. Right hypochondrium tender. Laboratory tests: glucose 55.6 mmol/l, WCC 23500/dl, CRP 189.6 mg/l, normal LFT. Blood gas: $H^+$ 69 mmol/, bicarbonate 4.9 mmol/l. Urine ketones + + + +. Diagnosed diabetic ketoacidosis & given IV insulin, fluids & antibiotics. Ultrasound scan of abdomen : acute cholecystitis. Anti GAD and beta islet cell antibodies negative. Random non fasting unstimulated C peptide 1769 pmol/l & insulin 66 mU/L. Total cholesterol 5.6 mmol/l.Converted to pre mixed insulin twice daily & metformin started when insulin requirement decreased. 1 year later, HbA1 c 7.2% without insulin.
**3:** Female aged 32 years had diarrhoea and vomiting at 34weeks gestation. Had IV fluids for 1 day, rapidly became dyspnoeic (oxygen saturation 95% on air) & tachycardic (110 beats/min). Blood pressure 133/70 mm/Hg, chest clear, heart sounds normal & no signs of deep venous thrombosis. Urine output > 50 ml/hour. BMI 35. Laboratory results :-WCC 17800/dl, urea 1.1 mmol/l, creatinine 81 µmol/l, Glucose 3.3 mmol/l. Urine ketones ++++, serum ketones 4 mmol/l. Blood gases: $H^+$ 82 nmol/l, $pO_2$ 11.5 kPa, $pCO_2$1.5 kPa, $HCO_3$ 3 mmol/l, base excess -23.5 mmol/l, $O_2$ sat 95%. Serum lactate 0.5 mmol/l, Random cortisol 953 mmol/l, drug & toxicology of screen of serum & urine negative & no growth on blood and urine culture including atypical serology. Chest x ray, echocardiogram, Doppler ultrasound of both legs & CT scan of chest & abdomen normal. Cardiotocogram: normally functioning foetus. On the basis of normal glucose, raised ketones & no other cause for extreme acidosis, euglycaemic diabetic ketoacidosis was diagnosed. Treated with IV insulin & 10% glucose to maintain blood glucose levels. She required 100 units of soluble insulin per day for 4 days to correct acidosis. Later converted to basal bolus regimen with humulin S (2 units tid) and I (4 units nocte). 1 week later oral glucose tolerance test (OGTT) result was in diabetic range. She had 2 normal fasting blood glucose levels at 26–28 weeks. Anti GAD and islet cell antibodies negative and random non stimulated C-peptide elevated at 3601 mmol/l. Basal bolus insulin continued until delivery by elective Caesarean section at 38 weeks of a normal healthy baby. OGTT 6 weeks postpartum normal.
**Result:** Ketoacidosis was presenting feature on diagnosis of T2DM.
**Conclusion:** Pathogenesis of ketoacidosis in T2DM is discussed and phenomenon of euglycaemia explained.

## 885

**Problems of analysis of the area under curve of glycemia in OGTT**

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**Background and aims:** For evaluation of results of standard oral glucose tolerance test (OGTT) is frequently used calculation of the areas under curve of glycemia in absolute values. Well known, that this parameter strongly correlates with HbA1 c level. But as at such calculations the formula implicitly includes the area under fasting glycemia, there is obscure a question, whether really postprandial glycemia correlates with HbA1 c.
**Materials and methods:** We carried out 84 standard OGTT at 42 patients with diabetes in dynamics. The following parameters were calculated: (a) the area under curve of glycemia (S); (b) the attitude of the area above a baseline level equal to the fasting glucose value (ignoring the area below fasting glucose) to general area S. Last calculation allows separate densities postprandial hyperglycemias in OGTT. Thus, influence of a level of fasting glycemia was excluded.
**Results:** At analysis are revealed positive correlations between HbA1 c and (a) the area under curve of glycemia in test ($r=0.448$, $p=0.00002$); b) the area above a baseline level equal to the fasting glucose value ($r=0.257$, $p=0.018$). Were no significant correlation between a HbA1 c level and the attitude of the area above a baseline level equal to the fasting glucose value to general area S ($r=-0.008$, $p=0.946$).
**Conclusion:** Thus, a level of fasting blood glucose in the greater degree is influenced the HbA1 c level. Postprandial hyperglycemia does not correlate with HbA1 c level.

## 886

**Does postprandial hyperglycemia really correlate with HbA1c?**

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**Background and aims:** Correlation between HbA1 c and absolute values (mmol/l) of postprandial glycemia (PPG) is well known. But such correlation simultaneously reflects dependence between fasting blood glucose (FBG) and HbA1 c, because FBG is implicitly entered in PPG. So it does not known if postprandial hyperglycemia really correlates with HbA1 c?
**Materials and methods:** We carried out 84 standard oral glucose tolerance tests (OGTT) in 42 diabetics in dynamics. For the proof of correlation between PPG and HbA1 c it was analyzed not only absolute values of a glycemia in OGTT, but also excess of a glycemia above FBG in percentage terms (EG).
**Results:** As well as others, we had shown significant correlation between: a) HbA1 c and FBG ($r=0.384$, $p=0.0003$); b) HbA1 c and PPG at 60 min ($r=0.405$, $p=0.0001$); c) HbA1 c and PPG at 120 min ($r=0.298$, $p=0.008$). Besides there was correlation between HbA1 c and an area under curve of glycemia in OGTT ($r=0.398$, $p=0.0002$). EG at 60 min of OGTT was $80.23 \pm 32.22\%$ and at 120 min – $90.99 \pm 25.82\%$. There was no correlations between HbA1 c and EG at 60 min ($r=0.267$, $p=0.810$) or 120 minutes ($r=0.054$, $p=0.624$). Accordingly there was no correlations between HbA1 c and an area under curve of glycemia in OGTT when such area was calculated as percent of FBG level ($r=-0.008$, $p=0.944$).
**Conclusion:** Our data show, that only permanently elevated level of a glycemia during a day (between meals and fasting values) raises HbA1c, but not accidentally and burst increase of PPG level. So there is arising unsolved question, whether is it necessary to achieve normalization of PPG levels, if it does not change HbA1 c? Especially because HbA1 c have been considered as a criterion of diabetes control and prognostic parameter for vascular diabetes complications.

## 887

**Self monitoring of blood glucose and glycaemic control in Type 2 diabetes**

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**Background and aims:** The cost of test strips used for self monitoring of blood glucose (SMBG) has increased dramatically in recent years and is in Sweden now exceeding 60 million Euro a year. However, the efficacy of SMBG in patients with type 2 diabetes has been questioned in some reports. The aim of this study was to explore the association between the use of SMBG and glycaemic control in patients with type 2 diabetes in primary health care.
**Materials and methods:** During November–December 2003, a cross-sectional observational study was conducted in 18 primary health care centres in Sweden where all known patients with diabetes mellitus were surveyed. Patients were categorised as type 1 or type 2 diabetes and were registered with respect to age, gender, treatment category, HbA1 c and number of visits to the health care centre. 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 HbA1 c.

**Results:** After exclusion of patients with type 1 diabetes and elderly patients living in nursing homes, 6665 subjects remained for further analyses. Thirty-one per cent (n=2081) were treated with diet only, 36% (n=2401) with oral agents and 33% (n=2183) were treated with insulin. In patients treated with diet only, 36% (n=741) were users of SMBG and the corresponding figures for subjects treated with oral agents and insulin therapy were 54% (n=1298) and 79% (n=1722), respectively. There were no difference in HbA1c between users (6.9%) and non-users (6.8%) of SMBG in patients treated with insulin or in patients treated with oral agents (6.3% both groups). In patients treated with diet only, users of SMBG had higher HbA1c compared to non-users (5.5% vs. 5.4%, p=0.002).
**Conclusion:** The use of SMBG was not associated with improved glycaemic control in any therapy category of patients with type 2 diabetes in primary care.

## 888

**A prospective study to assess the clinical application of glucose monitoring in hospitalised patients with Type 2 diabetes**
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**Background and aims:** To examine the clinical utility of in-patient blood glucose monitoring for type 2 diabetes in a UK district general hospital.
**Materials and methods:** Data was collected prospectively from 205 non-diabetes related emergency admissions. All patients had type 2 diabetes (Mean age 71yrs, 56% male, mean diabetes duration 10yrs). Patient demographics, type and frequency of glucose monitoring and action taken on low or high readings were recorded. Data was collected for day 1 and for days 3–6.
**Results:** 4% of admissions had new onset of type 2 diabetes. Even though 40% of patients had their usual treatment altered during admission only 7.3% were discharged with treatment that differed from pre-admission. Use of intravenous insulin infusion was common; 31% of all admissions on day 1 and 28% on days 3–6. Bedside blood glucose monitoring was performed 4 times daily or more in 37% of patients on day 1 and in 34% of patients in days 3–6 (excluding those on iv insulin infusion). None of the patients tested more than twice daily at home. The mean glucose value was 9.0 mmol/l on day 1 and 8.9 mmol/l on days 3–6. 19% of patients had a blood glucose value/s less than 3 mmol/l and this was actioned in 90% of cases by oral glucose.
73% of patents had a blood glucose value/s more than 10 mmol/l and no action was taken in 59%. 30% patients had a blood glucose value/s over 17 mmol/l; no action was taken in 28% however of those treated, 64% were prescribed an IV insulin infusion. No significant differences were seen between medical and surgical patients.
**Conclusion:** These results suggest that the current approach to in-patient glucose monitoring is unstructured and that a large number of patients are receiving intravenous insulin infusions. While hypoglycaemia seems to be managed well the treatment of hyperglycaemia could be improved. We propose that inpatient guidelines on blood glucose monitoring and treatment are necessary.

# PS 82

# Hypoglycaemia pathogenesis/ experimental

## 889

**Microinjection of diazoxide to the ventromedial hypothalamus augments glucose counterregulation during hypoglycaemia**
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**Background and aims:** Impaired hormonal counter-regulation effects as many as 50% of individuals with type 1 diabetes (TIDM) by 10 years disease duration, increasing their risk of suffering severe episodes of hypoglycaemia and providing a significant obstacle to intensive insulin therapy. The detection of incipient hypoglycaemia is thought to occur predominantly in the brain with glucose-sensing neurons in the ventromedial hypothalamus (VMH) probably playing a key role. The mechanisms by which the VMH is able to detect a change in blood glucose are unknown, but recent evidence suggests a role for ATP-sensitive potassium channels (K-ATP).
**Materials and methods:** To determine whether K-ATP channel openers (KCO) might play a role in VMH glucose-sensing and lead to an amplification of the counterregulatory hormonal response to hypoglycaemia 230.7 ng Diazoxide (N=7) or vehicle (1%DMSO, and NaOH to pH equivalent, in saline; N=8) were bilaterally microinjected into the VMH of awake chronically catheterized Sprague-Dawley rats. Immediately following microinjection a 90 minute (20 mU/kg/min) hypoglycemic clamp (2.8 mmol/l) was performed. Counterregulatory hormones were measured at baseline and during hypoglycaemia.
**Results:** During equivalent hypoglycaemia VMH-Diazoxide injected rats required less exogenous glucose (9.9 ± 2.5 vs. 19.2 ± 2.6 mg/kg/min; p<.05). This was associated with significant increases in plasma adrenaline (10.3 ± 1.5 vs. 3.7 ± 0.5 nmol/l; p<.05) and glucagon (195 ± 52 vs. 54 ± 6 ng/l; p<.05).
**Conclusion:** Direct delivery of the KCO Diazoxide to the VMH improves glucose counterregulation during acute hypoglycaemia in normal Sprague-Dawley rats. This suggests that: (i) K-ATP channels play a key role in hypoglycaemia sensing in the VMH, and (ii) KCO may offer therapeutic potential for individuals with type 1 diabetes who have defective glucose counterregulation.
*Supported by: Career Development Award from the JDRF*

## 890

**Renin/Prorenin ratio and rate of severe hypoglycaemia in Type 1 diabetes**
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**Background and aims:** We have previously shown a strong positive association between renin-angiotensin system (RAS) activity and rate of severe hypoglycaemia (SH) in type 1 diabetes. This study examines whether a similar association exists for activated negative feedback in the RAS.
**Materials and methods:** 171 consecutive patients with type 1 diabetes, untreated with ACE inhibitors or angiotensin II receptor antagonists, were followed for one year for episodes of SH (defined as episodes needing assistance from others). Plasma renin and prorenin were determined by an activity-based assay in the 156 subjects who had a blood glucose above 3.5 mmol/l at the time of sampling. Degree of negative feedback was assessed by renin/prorenin ratio (R/P).
**Results:** A significant inverse association between R/P and rate of SH (p=0.013) was found. Subjects with R/P in the lowest quartile had a relative rate (RR) of 4.5 (95% CL: 1.9–10.9) compared to those with R/P in the upper quartile (p=0.0008). Subjects with R/P in the second and third quartiles had intermediate RR of 2.6 (95% CL: 1.1–6.4) and 2.9 (95% CL: 1.2–7.1), respectively. There was no significant association between R/P and rate of mild (p=0.87) or biochemical hypoglycaemia (p=0.48) or other risk factors such as self-reported state of awareness (p=0.69), residual beta-cell function (p=0.25) or metabolic control (p=0.12).
**Conclusion:** Low renin/prorenin ratio, which is a sign of negative feedback in the renin-angiotensin system, is associated with high rate of severe hypoglycaemia. This is further evidence for an association between acti-

vated renin-angiotensin system and occurrence of severe hypoglycaemia in type 1 diabetes.
*Supported by: EFSD/JDRF/Novo Nordisk programme on Type 1 diabetes*

## 891

**High activity of Angiotensin-converting enzyme (ACE) and the DD-polymorphism of the ACE gene are associated with lower C-peptide levels in Type 1 diabetes mellitus**

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**Background and aims:** Decline in beta-cell-function in type 1 diabetes is known to result in increased frequency of hypoglycemic episodes as well as microvascular complications. The loss of beta-cell-function is measured by a stimulated C-peptide response and is predicted by the presence of islet antibodies (e. g. GAD). C-peptide is released from the beta-cell in amounts equimolar to insulin and was long thought to be biologically inactive. Recently, it has been shown that C-peptide has direct vascular effects as it increases nutritive capillary blood flow via stimulation of endothelial nitric oxide synthase (eNOS).
High activity of Angiotensin-converting enzyme (ACE) – genetically determined as deletion-genotype (DD) of the ACE-gene – is another vascular factor associated with reduced awareness of hypoglycemia in type 1 diabetes and increased risk of experiencing episodes of severe hypoglycemia.
The aim of this study was to predict the individual risk of severe hypoglycemia in newly diagnosed adult type 1 diabetes by correlating the C-peptide level to ACE-activity, several vascular polymorphisms (ACE, ATR1, eNOS), islet antibodies and the sympathoadrenergic reactability to hypoglycemia during an hypoglycemic clamp test.
**Methods:** 28 newly diagnosed type 1 diabetic patients were followed for 3 years. Every six months the endogenous C-peptide reserve was determined by an iv. glucagon-test. ACE-activity and insulin autoantibodies were measured in serum. Genotyping for polymorphisms was performed by PCR (ACE, eNOS) and nested PCR followed by restriction digestion (ATR1). 17 patients were clamped in hypoclycemia and epinephrine, norepinephrine and hypoglycemia symptoms were recorded.
**Results:** The distribution of the different genotypes was: 19% II, 53 % ID and 28 % DD for ACE, 67 % ins/ins, 33 % del/ins and 0% del/del for eNOS intron 4, 53% AA, 43% AC and 4 % CC for ATR1. The ACE-activity was twice in patients with the DD-genotype. Patients with the lowest C-peptide had the highest ACE-activity (p= 0.03) and a tendency towards lower epinephrine (p=0.1) as well as a tendency to reduced awareness of hypoglycemia (p=0.25) during hypoglycemic clamp. GAD positive individuals had significant lower levels of C-peptide (p= 0.002) and a tendency towards higher ACE-activity. Differences in eNOS or ATR1 genotypes did not change any of the measured parameters.
**Conclusion:** Patients with newly diagnosed adult onset type 1 diabetes with low C-peptide levels have high ACE-activity and might be more susceptible to hypoglycemia unawareness and reduction of sympathoadrenergic reactability as tested in a hypoclycemic clamp series. In clinical studies C-peptide serves as a marker of beta-cell function. Here we show that it deteriorates faster in individuals with genetically determined high ACE-activity. Alternatively C-peptide might exert direct vasodilatory effects in the microcirculation during hypoglycemia serving as a counterregulating tool to the ACE-system.
*Supported by: Fachbereich Medizin der Justus-Liebig-Universität Gießen*

## 892

**CYP2C9 slow metaboliser genotypes and sulphonylurea induced severe hypoglyacemia**

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**Background and aims:** Cytochrome P 450 (CYP) Enzyme 2C9 largely influences metabolism of oral antidiabetic drugs, in particular sulphonylureas. However, the effect of CYP2C9 induced differences in drug kinetics on the risk of severe hypoglycaemia (SH) has not been studied in diabetic patients yet.
**Materials and methods:** 20 patients with type 2 diabetes (age 74 ± 10 years; HbA1 c 6,5 ± 0,7%; creatinine-clearance 47 ± 28 ml/min.) who were admitted to hospital because of SH were genotyped for CYP2C9*2 and CYP2C9*3. Frequency of genotypes was compared to a sample of DNA from 336 Caucasian diabetic patients receiving oral antidiabetic drugs and without a history of SH , and to a large sample of 1988 healthy Caucasian volunteers. The hypoglycemic patients had been treated with sulphonylurea drugs glimepiride (n=17; daily dose 2.4 ± 1.4 mg) und glyburide (n=3; daily dose 9.3 ± 2 mg).
**Results:** Two (10%) of the 20 patients were carriers of the rare genotypes CYP2C9*2/*3 and CYP2C9*3/*3. Despite the small number of cases, the frequency of the slow metabolizer genotype differed significantly from the population frequency of 2.1% (41/1988) observed in 1988 Caucasians (p=0.02) and in 336 diabetic patients without history of SH (7/336, p=0.028) of whom, 7 were slow metabolizers. The remaining 18 patients did not show an overrepresentation of certain genotypes. Especially, the heterozygous genotypes CYP2C9*1/*2 and CYP2C9*1/*3 were not found more frequently than expected in Caucasians. No individual with the rare genotype CYP2C9*2/*2 was found.
**Conclusion:** Other risk factors than CYP2C9 decreased activity seem be responsible for the overwhelming part of SH in this sample. However, a certain part of SH might be explained by CYP2C9 genotypes predicting low enzyme activity. Interestingly, the heterozygous genotypes carrying still one wildtype allele were not found in higher frequency than in the normal population indicating that these individuals still have enough CYP2C9 activity to cover an elevated risk for sulphonylurea induced SH.

## 893

**Cardiac autonomic function during insulin induced hypoglycaemia in subjects with Type 2 diabetes mellitus**

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**Background and aims:** Little is known about effects of hypoglycaemia on cardiovascular autonomic function in type 2 diabetes subjects. To clarify the effect of hypoglycaemia on cardiovascular autonomic function in type 2 diabetic subjects, we performed hyperinsulinaemic hypoglycaemic clamp studies.
**Materials and methods:** Cardiac autonomic neuropathy was excluded in each subject at screening.We performed stepped hyperinsulinaemic hypoglycaemic clamp studies on 4 subjects (3 male, 1 female) with T2DM (mean ± SD Age 51.3 ± 7.4 years, HbA1 c 7.7 ± 1.2 %, body mass index 28.0 ± 2.0 kg/m$^2$ and diabetes duration 6.0 ± 1.3 years). Primed continuous insulin infusion (3 mU/kg/min) was administered for 180 minutes. Glucose levels were lowered from baseline (5–7 mM) to 5.0, 3.2, 2.6 and back to 5 mM at 45 minute intervals and counterregulatory hormones measured at each glucose plateau. Beat to beat BP and HR were monitored using Finometer TNO (TPD Biomedical Instrumentation). Cardiovascular autonomic tests (deep breathing at 6 breaths per minute, valsalva manoeuvre and tilting to 85° for 6 minutes) were performed at each glucose plateau.
**Results:** Data are presented in order of baseline glucose, 5.0, 3.2, 2.6 and 5.0 mM. Heart rate variability during deep breathing were 15.9 ± 9.3, 16.6 ± 5.3, 14.8 ± 5.8, 8.1 ± 1.6 and 14.4 ± 6.1 beats per minute respectively (p = 0.024 at initial glucose 5.0 vs. 2.6 mM). Mean BP fall in phase II (early) during valsalva manoeuvre were observed at each glucose plateau: 17.5 ± 5.1, 30.5 ± 5.1, 31.8 ± 10.5, 24.3 ± 13.6 mmHg respectively (p = 0.01 at glucose 5.0 vs. 3.2 mM, p = 0.05 at glucose 5.0 vs. 2.6 mM). Mean BP deficit from baseline BP in phase II (late) was absent at baseline glucose. Mean BP deficit were observed at subsequent glucose plateaux (5.0, 3.2, 2.6 and 5.0 mM): 7.5 ± 5.2, 17.5 ± 15.2, 28.3 ± 15.3 and 13.5 ± 13.0 mmHg respectively (p = 0.04 at glucose 5.0 vs. 2.6 mM). Plasma epinephrine levels during each glucose plateau (baseline, 5.0, 3.2, 2.6 and 5.0 mM) are as follows: 0.04 ± 0.03, 0.24 ± 0.20, 0.96 ± 0.29, 3.06 ± 0.89, 0.46 ± 0.35 nM (p=0.006 at initial glucose 5.0 mM vs. 2.6 mM).
**Conclusion:** In subjects with T2DM, hypoglycaemia during glucose 2.6 mM is significantly associated with reduced heart rate variability during deep breathing and defect in BP responses during valsalva manoeuvre in phase II (early and late) has been observed during hypoglycaemia. This preliminary data may suggest that in subjects with Type 2 diabetes, cardiac autonomic function during insulin induced hypoglycaemia is altered possibly due to both parasympathetic and sympathetic effects.

## 894

**Measurement of brain perfusion in response to acute hypoglycaemia in healthy volunteers: a 15O-water positron emission tomography study**

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**Background and aims:** Positron emission tomography (PET) with the tracer $^{15}$O-labelled water can be used to quantify regional and whole brain perfusion and to indicate regional neuronal activation in man. The aim of this study was to quantify whole brain perfusion in response to acute moderate hypoglycaemia and to identify regions showing different responses or neuronal activation.
**Materials and methods:** Eight healthy men underwent a hypoglycaemic insulin clamp, plasma glucose 2.6 mmol/l, over a 90 minute period. For each subject, four $^{15}$O-water PET scans were performed at 30 min intervals. The first scan was performed prior to hypoglycaemia and the subsequent scans at 0, 30 and 60 min into the hypoglycaemic plateau. Each PET scan comprised a dynamic acquisition lasting six minutes, and arterial blood samples were drawn during each scan. Whole brain data was analysed using well established methods to obtain perfusion measurements in absolute units of ml/100g/min. For each scan images were also summed over the complete 6 min acquisition period and analysed using the Statistical Parametric Mapping (SPM) technique to identify regional changes.
**Results:** Average whole brain perfusion did not change significantly between the measurements made at baseline and during hypoglycemia, or throughout the period of hypoglycemia (Baseline: mean 39.14 ± s.d. 4.32; t = 0: 40.11 ± 4.47; t=30: 42.46 ± 5.10 and t = 60: 37.57 ± 6.67 mls /100 g/min, p=NS).
Regional analysis with SPM highlighted an area of brain in the midline, anterior and inferior to the 3$^{rd}$ ventricle, which included sublobar regions of the cerebrum, the corpus callosum, limbic lobe, anterior cingulate, lentiform nucleus and putamen (Z-score=4.81, F=20.75). The region was associated with an increase in blood flow over the duration of the four scans.
**Conclusions:** Using well validated methods we have been unable to detect any change in average whole brain perfusion in response to acute hypoglycaemia in healthy men. A single region of increased perfusion was identified. The significance of this region is not currently clear but the increased perfusion is likely to indicate neuronal activation in this area.

## 895

**Sensitivity of the $\beta_2$-adrenergic receptor in patients with Type 1 diabetes and hypoglycemia unawareness**

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**Background and aims:** Hypoglycemia unawareness is characterized by a typical loss of autonomic warning symptoms before development of neuroglycopenia, often accompanied by an attenuated adrenaline response to hypoglycemia. Based on an impaired heart rate (HR) response to infusion of the nonselective β-adrenergic agonist isoproterenol, it has been suggested that reduced β-adrenergic sensitivity plays a role in hypoglycemia unawareness. In the present study, we investigated whether this reduced β-adrenergic sensitivity confers to the $\beta_2$-adrenergic receptor.
**Materials and methods:** $\beta_2$-adrenergic sensitivity was determined in 10 type 1 diabetic patients with hypoglycemia unawareness (DM-Unaware), 12 type 1 diabetic patients with intact hypoglycemic awareness (DM-Aware), and 11 healthy controls (CON) by measuring the forearm blood flow (FBF) response to intraarterial salbutamol, a selective $\beta_2$-adrenergic agonist. Salbutamol was infused into the brachial artery in 6 increasing doses ranging from 0.003 to 1.0 µg/min/dL. FBF was measured in the infused and in the contralateral arm by venous occlusion plethysmography. Diabetic patients received low dose insulin prior to FBF measurements to ensure that all experiments were carried out under normoglycemic (glucose <7.0 mmol/L) conditions.
**Results:** At baseline, FBF was 1.9 ± 0.3, 1.5 ± 0.2, and 2.2 ± 0.4 mL/min/dL in CON, DM-Unaware, and DM-Aware, respectively ($P$ = NS). In response to salbutamol, FBF increased 9-fold in CON, 10-fold in DM-Unaware, and 8-fold in DM-Aware ($P$ = NS). The highest salbutamol dose generated systemic effects in all groups, but not in similar fashion. In CON and DM-Aware, FBF increased in the contralateral arm by an average 47 ± 11 % ($P < 0.03$) and HR increased by 13 ± 1 bpm ($P < 0.001$), whereas mean arterial pressure (MAP) remained unchanged. In DM-Unaware, the highest salbutamol dose failed to affect FBF in the contralateral arm, and caused a modest increase in HR (+8 ± 2, $P = 0.004$) and a fall in MAP (−8 ± 2 mmHg, $P = 0.03$).
**Conclusion:** The sensitivity of the $\beta_2$-adrenergic receptor is not reduced in diabetic patients with hypoglycemia unawareness. However, the disparate systemic effects of the highest salbutamol dose in diabetic patients with hypoglycemia unawareness compared to controls and diabetic patients with intact awareness suggests an association between hypoglycemia unawareness and reduced $\beta_1$-adrenergic sensitivity.

# PS 83

# Hypoglycaemia: epidemiology and monitoring

## 896

**Severe hypoglycaemic episodes in a Type 1 diabetic cohort. A descriptive study**

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**Background and aims:** To describe occurrence and characteristics of severe hypoglycaemia (SH) in a well-defined cohort of type 1 diabetic patients.
**Materials and methods:** A cohort of 213 consecutive patients with type 1 diabetes was followed for one year by monthly questionnaires and immediate reporting of episodes of SH (defined as episodes needing assistance from others). A total of 237 episodes of SH occurred during the period. All incidents were thoroughly validated in structured telephone interviews performed within 24 hours after the event and characterised by place and time of day, severity and circumstances including cause of the episode and cause of the lacking self-management.
**Results:** SH occurred most commonly at night (37%) and in the afternoon (24%). There were no significant differences in number of events on weekends and weekdays. Most (70%) of the incidents took place at home and only 7% occurred at work. Supposed reasons for SH were physical activity (32%), insufficient intake of calories (17%), excess insulin dose (8%), and other specified explanations (13%) (9% of the patients reported two or more possible reasons). In 45% of the events there was no obvious reason for the incident. Activities immediately before onset of SH were sleeping (44%), relaxing (16%), doing sports (3%), partying (2%), working (16%), and daily activities (19%). Reasons for not acting adequately when awake were no recognition of hypoglycaemic symptoms (43%), recognition of symptoms but too little time to react (34%), misinterpreting or ignoring symptoms (18%), and „other reasons" (5%). Among incidents occurring at home when awake, 45% were associated with symptoms and 55% were not, whereas 71% of the incidents occurring outside home were associated with symptoms and 29% were not ($p<0.005$). The severity of the episodes as indicated by loss of consciousness, convulsions, level of blood glucose, length of recovery was the same at home and outside home. More patients were treated with glucagon at home than outside home (17% vs. 4%; $p=0.001$).
**Conclusion:** Most episodes of severe hypoglycaemia in type 1 diabetes occur when sleeping or relaxing at home and only few at the place of work. Almost half of the episodes are unexplained. At home, most episodes occurring when awake are not recognised whereas those appearing at other places are mostly perceived but misinterpreted or mistreated.

## 897

**Evaluation of the impact of hypoglycaemia on health care resource use, productivity, fear of hypoglycaemia and health utility in a UK population**

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**Background and aims:** Hypoglycaemia is a common adverse event of diabetes treatments. In addition to direct symptoms, hypoglycaemia adversely affects people in many ways, it can cause fear and anxiety and is likely to increase health care resource use and impact productivity. The purpose of this study was to evaluate these events as a function of hypoglycaemia severity and frequency.
**Materials and methods:** A postal survey mailed to 3,500 subjects with diabetes identified through hospital review, included questions on the frequency and impact of hypoglycaemia, health care resource use, diabetes management, lifestyle, the EQ-5D and the Hypoglycaemia Fear Scale (HFS). Self-reported hypoglycaemic events were classified as mild, moderate, nocturnal or severe. Detailed phenotypic details were available through the Health Outcomes Data repository (HODaR). Events were measured over a six-week period.
**Results:** At this analysis point, 729 patients have responded to the survey (20.8%). Of the respondents, 62.3% have Type 2 diabetes and 59.0% were male. The mean event rates and the mean utility and HFS score are listed in the table.
**Conclusion:** There were generally consistent associations between both severity and frequency in the parameters quantified, indicating that hypoglycemia has a high negative impact on quality of life, productive activities and health care resource use.

| | Severe | Moderate | Nocturnal | Mild | 0-hypo's | >=2 | >2<=6 | >6 |
|---|---|---|---|---|---|---|---|---|
| Subjects (n) | 56 | 164 | 174 | 283 | 223 | 112 | 161 | 180 |
| Mean times visit GP | 1.6 | 1.1 | 1.0 | 1.0 | 0.8 | 1.0 | 1.1 | 1.0 |
| Mean times visit Practice Nurse | 2.1 | 0.8 | 0.8 | 0.7 | 0.6 | 0.9 | 0.7 | 1.1 |
| Mean times Nurse visited home | 1.0 | 0.2 | 0.1 | 0.3 | 0.3 | 0.1 | 0.4 | 0.4 |
| Mean times visited by Social Services | 1.1 | 0.2 | 0.3 | 0.2 | 0.6 | 0.0 | 0.2 | 0.4 |
| Mean Days off Work | 1.6 | 0.8 | 1.0 | 0.5 | 0.4 | 0.3 | 0.9 | 0.8 |
| Mean Days off Hobby | 4.5 | 6.1 | 4.8 | 2.6 | 3.3 | 2.3 | 3.6 | 4.5 |
| Mean Days Helped | 10.8 | 8.7 | 7.1 | 6.8 | 5.7 | 4.2 | 8.6 | 7.4 |
| All productive events | 20.5 | 16.5 | 14.0 | 10.8 | 10.6 | 7.8 | 14.2 | 14.8 |
| Mean utility (EQ-5D) | 0.52 | 0.60 | 0.63 | 0.67 | 0.73 | 0.71 | 0.64 | 0.61 |
| Mean Hypo' Fear Score | 17.8 | 15.1 | 14.4 | 9.2 | 3.5 | 9.6 | 11.3 | 14.4 |

## 898

**The economic and quality of life impact of hypoglycaemia: results of a Swedish study**

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**Background and aims:** Hypoglycaemia is an often under-recognized side effect of Type 2 diabetes therapy. This study assessed the burden of hypoglycaemia in patients with Type 2 diabetes aged ≥35 years who had been treated with insulin or oral antidiabetic agents.
**Materials and methods:** Patients were divided into those who had reported hypoglycaemic symptoms during the past month (n=115) and those who had not (n=194). The mean age was 65 ± 11 years and mean baseline $HbA_{1c}$ was 6.8 ± 1.2%. Health-related quality of life (HRQoL) data were assessed using 3 surveys: EuroQoL EQ-5D, questions related to general wellbeing from the Swedish Study of Living Conditions and a modified form of the Hypoglycaemia Fear Survey. Surveys were administered once to each patient. Demographics, prevalence and severity of hypoglycaemic events, treatment details and $HbA_{1c}$ values were collected from medical records. Direct healthcare costs (resource use for medical visits and inpatient care) and indirect costs (decreased productivity) were measured.
**Results:** Patients with hypoglycaemic symptoms reported lower general health, had more fears and worries of hypoglycaemia and thought more about their diabetes compared with patients without such symptoms. Direct and indirect costs of hypoglycaemia were estimated to be 98 Swedish Kronor (SEK) and SEK107, respectively, per patient with hypoglycaemia, for a 1-month period, giving a total 1-month cost of SEK76 per patient with Type 2 diabetes.
**Conclusion:** Hypoglycaemia is a common problem for many patients with Type 2 diabetes, affecting health and impacting on daily life. A reduction in hypoglycaemia, without reducing glycaemic control could, therefore, potentially improve patients' health and lead to cost reductions.
*Supported by: Aventis.*

## 899

**High prevalence of impaired hypoglycaemia awareness is detected in Type 1 diabetes mellitus patients using continuous glucose monitoring**

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**Background and aims:** Hypoglycaemia is the main impediment to intensive glycaemic control in type 1 diabetes patients. For adjustment of insulin doses, we rely on self-reporting of hypoglycaemia by patients who use capillary blood glucose tests which provide limited information about daily glycaemic excursions. We therefore examined continuous interstitial fluid glucose profiles in patients (n=24) with type 1 diabetes mellitus to detect frequency of hypoglycaemia and correlation with patient detection of such events.
**Materials and methods:** We used the MiniMed Continuous Glucose Monitoring System (CGMS) to record interstitial fluid glucose continuously for 72 hours and analysed the data using manufacturer's software. The patients were selected consecutively in a diabetes clinic and appropriate consent was obtained. We defined hypoglycaemia as any glucose reading of less than 4.0 mmol.l$^{-1}$ for the purposes of the study.
**Results:** Analysis revealed that 10.3% of all glucose readings from our patients were in the hypoglycaemic range. 56% of such low readings were recorded at night. Patients recognised only 27% of hypoglycaemic events, thus 73% of recordings below 4 mmol.l$^{-1}$ (p=0.001) were undetected by patients. Further analysis revealed that eight patients had complete lack of hypoglycaemia awareness. Comparing this group (Group 1) with the group who retained awareness of hypoglycaemia (Group 2), we found no significant differences in: age (45 versus 39.25 years, p=0.16); duration of diabetes (12.1 versus 10.6 years, p=0.3); systolic blood pressure (136 versus 131 mm Hg, p=0.19); area under the curve for glucose over the three days (701.5 versus 662.6 mmol.hours/litre, p=0.7) or glycaemic control (HbA1 c 9.74% versus 8.79%, p=0.1).
**Conclusion:** Our results confirm the high prevalence of impaired hypoglycaemia awareness, especially nocturnal, in type 1 diabetes patients and indicate that continuous glucose monitoring may identify such patients prior to optimising their control.

## 900

**Hypoglycemia detection with two different continuous glucose monitoring systems**

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**Background and aims:** Continuous glucose monitoring is usefull in uncovering patterns in glucose levels at times when users normally don't measure blood glucose. Especially the detection of hypoglycemic episodes is important.
**Materials and methods:** We performed a study to evaluate blood and tissue glucose traces. Tissue glucose was measured with a Minimed Medtronic Sensor CGMS (one value every 5 minutes) and with two SCGM sensors, in development by Roche Diagnostics (one value every minute). The calibration was based on capillary blood glucose values (Akku-Chek Active), the SCGM device was calibrated 1–2 times a day, the CGMS device at least 4 times a day. The venous blood glucose was monitored at least every hour with the Accu-Chek Compact. Values below 50 mg/dl were measured twice to verify the value. We compared the tissue glucose, measured before and after the venous glucose value (due to the CGMS 5 minute intervals). The means of these two tissue values (of the CGMS sensor and the two SCGM devices) were compared with the mean of the venous glucose values below 50 mg/dl. Thirty-six experiments were performed and we evaluated a total of 1404 hours.
**Results:** Twelve patients with insulin pump therapy participated in the study (6 m, 6f, age 40.1 ± 6.0 [mean±standard deviation], diabetes since 25 ± 9.1 years, pump since 8.2 ± 6.4 years, HbA1 c 6.7 ± 0.8%). During 1404 hours we observed 12 hypoglycemic episodes for which all three measurements are available: Venous blood glucose 47.1 ± 2.2 mg/dl (mean±SD, n=12), SCGM 48.2 ± 8.6 mg/dl (n=24), CGMS 56.6 ± 12.9 mg/dl (n=12). All venous blood glucose values (100%), 14 of the 24 SCGM values (58.3%) and 3 of the 12 CGMS values (25%) were lower than 50 mg/dl. Between 50 and 70 mg/dl the SCGM´s measured 10 values (n= 24, 41.7%) and the CGMS measured 7 (n=12, 58.3%) values. None of the 24 SCGM mean values were above 70 mg/dl (0%), but 2 of 12 CGMS mean values (16.7%).
**Conclusion:** This results show that tissue glucose monitoring with different devices and methods is nearly comparable to venous BG measurements in hypoglycemic situations. Nevertheless some of the tissue glucose values are higher, so that the number of hyopglycemias could be underestimated. Higher warning levels could be helpful.

## 901

**Usefulness of continuous glucose monitoring system in detection of hypoglycemic episodes in various methods of intensive insulin therapy**

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**Background and aims:** Multiple doses insulin injection (MDI), intravenous insulin infusion (IVII) and continuous subcutaneous insulin infusion (CSII) are methods of short-term intensive insulin therapy (IIT), helpful in achieving good metabolic control in poorly controlled diabetic patients. However, IIT is associated with occurrence of side effects - hypoglycemic episodes. Having continuous glucose monitoring systems (CGMS) it is possible to precisely assess the number and duration of hypoglycemia. The aim of the study was to determine symptomatic and symptom-free hypoglycemic episodes of MDI, IVII and CSII on the ground of glucose measurements achieved from the CGMS (MiniMed Technologies) in hospital conditions.
**Materials and methods:** The study consisted of 90 poorly controlled type 2 diabetes, insulin-treated patients (mean age 61.4+/–4.2 years, duration of diabetes 8.5+/–2.2 years, glycated hemoglobin A1 c 9.8+/–1.6%), who were randomly divided into three identically numerical groups depending on treatment modalities: MDI, IVII or CSII. CGMS was used for 72 hours during IIT. Symptomatic and symptom-free hypoglycemic events (blood glucose < 3.5 mmol/l) were noted.
**Results:** Number of the symptomatic hypoglycemic episodes per day in MDI patients was almost twice as high as in CSII and IVII patients: 1.1, 0.59 and 0.66 event per one person, respectively (MDI vs. IVII, p<0.001; MDI vs. CSII p<0.001). The incidence of symptom-free episodes was in MDI about twice as high as in CSII and IVII: 0.46, 0.22 and 0.26 event per one person, respectively (MDI vs. IVII, p<0.001; MDI vs. CSII p<0.001). Mean duration of symptomatic hypoglycemia was 49 min in MDI, 35 min in IVII and 37 min in CSII (MDI vs. IVII, p<0.05; MDI vs. CSII p<0.05). Mean duration of symptom-free hypoglycemia was 35 min in MDI, 23 min in IVII and 20 min in CSII (MDI vs. IVII, p<0.05; MDI vs. CSII p<0.05). The statistical differences in number and duration of hypoglycemic episodes between MDI and CSII, MDI and IVII were observed. The IVII and CSII groups were statistically comparable (NS).
**Conclusion:** Basing on the glucose measurements from CGMS we conclude, that MDI is related both with the most numerous and with the longest hypoglycemia, including symptom-free hypoglycemia. Therefore, MDI, in comparison with CSII and IVII, is the least safe method of short-term intensive insulin therapy used in poorly controlled type 2 diabetes patients.

## 902

**Acute hypoglycaemia does not interfere with memory consolidation of previously learned material in healthy adults**

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**Background and aims:** Memory function is impaired during acute hypoglycaemia. Several pharmacological and physiological interventions have been shown to affect consolidation of learned material into long-term memory. The hippocampus and frontal cortex, areas that are critical to long-term memory, are known to be sensitive to severe hypoglycaemia. It is possible that a period of acute hypoglycaemia may disrupt consolidation of previously learned material, so that subsequent recall is impaired. This would imply an anterograde amnesic effect of hypoglycaemia.
**Materials and methods:** Sixteen healthy volunteers each underwent one hypoglycaemic and one euglycaemic clamp. During each session there was a learning phase during euglycaemia (blood glucose 4.5 mmol/l), during which subjects viewed, and attempted to memorize, 40 pictures of faces presented in sequence, followed by 50 words. The experimental condition (hypoglycaemia at 2.5 mmol/l, or euglycaemia) followed for one hour, after which the clamp was discontinued. Approximately 2.5 hours after exposure, recall was tested during uncontrolled normoglycaemia. For both faces and words, previously-viewed stimuli (targets) and decoys were presented randomly, and subjects were required to identify each test stimulus as new or old.
**Results:** Corrected scores for face identification were similar following euglycaemia and hypoglycaemia (67.2 ± 8.2% and 66.9 ± 7.4% respectively;

p=0.9), as were scores for word identification (78.0 ± 8.0% and 77.1 ± 12.12% respectively; p=0.7).
**Conclusion:** Acute, moderate hypoglycaemia induced soon after learning did not impair formation of long-term memory. The consolidation process may have been rapid, and therefore complete before hypoglycaemia was achieved, or may only be affected at lower blood glucose levels.
*Dr R Warren was supported by independent funding from Eli Lilly & Company.*

## 903

**Relationship between nocturnal hypoglycaemia and morning blood glucose in Type 1 diabetes**
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**Background and aims:** The relationship between nocturnal hypoglycaemia and morning blood glucose concentration is still debated. This is probably due to technical limitations in recording of nocturnal hypoglycaemia. We assessed the relationship by means of continuous subcutaneous glucose monitoring, which offers improved possibility of recording nocturnal hypoglycaemia in real life.
**Materials and methods:** One hundred and eighteen patients with type 1 diabetes (median age 54 (range 24–74), duration of diabetes 26 years (range 7–54), HbA1 c 8.3 % (range 5.8–10.8) and 90% on multiple injection therapy) underwent 6 days of continuous subcutaneous glucose monitoring with Medtronic MiniMed Continuous Glucose Monitoring System (CGMS). HemoCue blood glucose determinations were used for calibration. Participants completed a detailed diary documenting all meals and snacks, insulin doses and episodes with symptoms of hypoglycaemia. Data from all nights with valid CGMS readings, and calibration values entered at bedtime (between 21.00 and 02.00) and fasting in the morning (between 05.00 and 10.00) were identified. Nights (between bedtime calibration value and morning calibration value) were classified as hypoglycaemic if CGMS readings were 2.2 mmol/l for at least 10 minutes or non-hypoglycaemic if nadir CGMS readings were > 3.5 mmol/l. Nights with CGMS nadir between 2.2 and 3.5 mmol/l were discarded. Primary end point was fasting morning blood glucose measured by HemoCue.
**Results:** A total of 413 nights were classified as either hypoglycaemic (27%) or non-hypoglycaemic (73%). Fasting morning blood glucose was significantly higher after non-hypoglycaemic nights when compared to hypoglycaemic nights (11.5 vs. 6.1 mmol/l, p<0.0001). In case of a morning blood glucose value > 15 mmol/l the probability of having had a hypoglycaemic episode the preceding night was 3% (95% CI 1–12%). Conversely, in case of a morning blood glucose < 5 mmol/l the probability of having had a hypoglycaemic episode the preceding night was 80% (95% CI 69–89).
**Conclusion:** The still widespread belief that high morning blood glucose may indicate nocturnal hypoglycaemia the preceding night (the Somogyi effect) is rejected by these data based on CGMS recorded hypoglycaemic events in patients with type 1 diabetes.
*Medtronic provided sensors for the study.*

## 904

**Risk of glucose meters not detecting hypoglycemia due to interfering substances in blood**
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**Background and aims:** Accuracy varies among glucose meters. Some systems are interfered by certain substances in the blood. We evaluated the effects of two common substances – paracetamol and uric acid – on two glucose-monitoring systems based on amperometric electrochemical technologies. Paracetamol (acetaminophen) is a common medication for pain relief; uric acid is known to be at increased concentrations in the blood in gout and renal failure, a common long-term complication of uncontrolled diabetes.
**Materials and methods:** Venous blood samples were collected before breakfast from healthy subjects who had not taken any medications for at least 24 h before blood collection. Two to four hours after collection, the blood samples had glucose concentrations around 65 mg/dL (3.6 mmol/L) and were used in the studies. In the dose-response study, varying concentrations of paracetamol (up to 14 mg/dL or 927 µmol/L) or uric acid (up to 14 mg/dL or 0.83 mmol/L) were added to aliquots of the venous blood sample and then each aliquot was tested 10 times with two glucose monitoring systems based on electrochemical technology. Meter A uses glucose dehydrogenase (GDH-PQQ) on the test strip, which requires 4 µL of blood and 26 seconds for a test. Meter B uses glucose dehydrogenase (GDH-NAD) on the test strip, requiring 1.5 µL of blood and 10 seconds for a test. Meters A and B apply potentials of 300 mV and 200 mV, respectively to drive the electrochemical reaction on the test strip. In the additive-effect study, paracetamol (4 mg/dL; 265 µmol/L) and uric acid (9 mg/dL; 0.53 mmol/L), alone and in combination, were added to aliquots of the venous blood sample and then each aliquot was tested 10 times with the 2 monitoring systems. Results were compared to the control blood aliquot with no substance added. With therapeutic doses, blood paracetamol levels up to 4 mg/dL have been reported. Blood uric acid levels above 9 mg/dL are common in renal failure.
**Results:** In the dose-response study, results of Meter A increased as a function of the concentrations of paracetamol and uric acid, with maximum biases of 36% and 22%, respectively. Results of Meter B did not change significantly (maximum biases of 2%; p>0.1). In the additive-effect study, paracetamol (4 mg/dL; 265 µmol/L), uric acid (9 mg/dL; 0.53 mmol/L) and their combination caused significant biases (p<0.002) of 8%, 25% and 30%, respectively with Meter A. Adding paracetamol, uric acid and their combination to a hypoglycemic blood sample (65 mg/dL; 3.6 mmol/L;) caused Meter A to report euglycemic values of 70, 82 and 85 mg/dL (3.9, 4.6 and 4.7 mmol/L), respectively. These substances did not cause clinically significant changes in Meter B results (the combination of paracetamol and uric acid produced a bias <7%).
**Conclusion:** Different glucose monitoring systems respond differently to interfering substances. Meter A showed interference, presumably due to the higher applied potential triggering nonspecific reactions with substances such as paracetamol and uric acid. Users should read the test strip insert (instructions for use) carefully to understand the limitations of their glucose monitoring system, especially if there is a risk of not detecting results of hypoglycemia.

# PS 84

# Hypoglycaemia: clinical aspects

## 905

**Effect of glimepiride and its hydroxy metabolite (M1) for severe glimepiride induced hypoglycaemia**

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**Background and aims:** There are no essential differences in respect to the clinical characteristics or time course of glibenclamide vs. glimepiride induced severe hypoglycaemia (SH), even protracted courses of SH have been reported in glimepiride induced SH. Thus, we determined the diagnostic value and the time course of the hydroxy metabolite of glimepiride (M1) during SH associated with glimepiride therapy in order to examine a possible causal role of M1 in prolonged SH.
**Materials and methods:** In nine type 2 diabetic patients (age 81 ± 9 [65–93] yrs; diabetes duration 9 ± 4 [3–15] yrs; initial blood glucose 33 ± 16 [10–54] mg/dl (1.8 ± 0.9 mmol/l); HbA1 c 7.2 ± 1.1 [5.6–8.7] %; creatinine clearance 49 ± 33 [15–107] ml/min) who experienced glimepiride associated SH with neuroglucopenic presentation, M1 serum concentrations were determined by a validated atmospheric pressure chemical ionization liquid chromatography-mass spectrometry (APCI-LC-MS) assay in blood samples taken at 4-h intervals prior to and during treatment with glucose i.v.
**Results:** Whereas initially glimepiride levels were above the limit of detection (LOD <0.01 mg/l) in only 4 cases (45%), M1 concentrations could still be detected in all nine cases of SH associated with glimepiride therapy. Generally, M1 was detectable 4–8 h longer than glimepiride. Even in three protracted courses of SH requiring > 12 h of i.v. glucose administration there was no significant accumulation of M1.
**Conclusion:** Determination of M1 in serum allowed us to obtain indications of glimepiride associated hypoglycaemia for a longer time than determination of the parent compound. However, it is questionable whether M1 is a major factor contributing to prolonged courses of glimepiride associated SH.

## 906

**Relationship between medically recorded hypoglycaemic event rates and $HbA_{1c}$ levels in patients initiated on long-acting insulin (glargine) or intermediate-acting insulin (NPH) within a managed care population**

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**Background and aims:** To compare differences in medically recorded hypoglycaemic event rates identified in a medical claims database with respect to $HbA_{1C}$ values within a managed care population newly initiated on insulin glargine or NPH insulin.
**Materials and methods:** This was a retrospective cohort study performed using a 2.5 million member southeastern US managed care integrated medical and pharmacy administrative claims and laboratory results database. All patients newly initiated (no insulin use in prior 4 months) on insulin glargine or NPH insulin between 7/1/00 and 8/31/02 were identified. Hypoglycaemic events were identified in the medical claims via ICD-9 codes. Negative binomial regression model techniques were utilized to predict hypoglycaemic events. Model covariates included age, gender, baseline hypoglycaemic events, regular insulin use, oral hypoglycaemic agents and lowest $HbA_{1C}$. The hypoglycaemic event rate was generated from the model by varying $HbA_{1C}$ while holding other covariates constant.
**Results:** There were 1434 patients identified (insulin glargine=310, NPH insulin =1124). Mean age was 53 ± 17 years; 52% were male; mean treatment duration was 9 ± 4 months. A total of 88 hypoglycaemic events were observed. The given hypoglycaemia event rate was lower at all $HbA_{1c}$ values for insulin glargine compared with NPH insulin (Table). Patients newly initiated on NPH insulin, compared with insulin glargine, were more likely to be associated with a higher hypoglycemic event rate (IRR 3.18, 95% CI: 1.33 to 7.62).
**Conclusion:** Patients newly initiated on insulin glargine had a lower medically recorded hypoglycaemic event rate at a given $HbA_{1C}$ compared to those newly initiated on NPH insulin.

| $HbA_{1c}$ (%) | Hypoglycaemia (event-rate/100 patient-years) | |
|---|---|---|
| | Insulin glargine | NPH insulin |
| 4 | 10.95 | 29.2 |
| 6 | 10.95 | 21.9 |
| 7 | 7.3 | 18.3 |
| 8 | 7.3 | 18.25 |
| 10 | 7.3 | 14.6 |
| 11 | 7.3 | 14.6 |
| 13 | 3.65 | 10.95 |
| 14 | 3.65 | 10.95 |
| 15 | 3.65 | 7.3 |
| 16 | 3.65 | 7.3 |

*Supported by: Aventis Pharma.*

## 907

**Lower risk of nocturnal hypoglycaemia with insulin detemir versus NPH insulin in people with diabetes: a meta-analysis of controlled phase III trials**

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**Background and Aims:** Results from several clinical trials indicate that the long-acting insulin analogue, insulin detemir (IDet), is at least as effective as NPH insulin in maintaining glycaemic control, with a significantly lower risk of nocturnal hypoglycaemia and less day-to-day variation in fasting blood glucose levels. To substantiate the generality of these findings, the risk of hypoglycaemia with insulin detemir versus NPH insulin was compared in a meta-analysis of 6 phase III trials in people with Type 1 and Type 2 diabetes.
**Materials and Methods:** All trials were multinational, randomised, open-label, parallel group trials comparing insulin detemir and NPH insulin in a basal-bolus regimen in people with Type 1 diabetes (IDet: 1336; NPH: 814) or Type 2 diabetes (IDet: 536; NPH: 363) using regular human insulin or rapid-acting insulin aspart before meals. Baseline demographic characteristics were well balanced between treatments, and were generally representative of the respective patient populations. The relative risk of nocturnal hypoglycaemia (23:00 to 6:00 h) and hypoglycaemia over 24 h with insulin detemir versus NPH insulin was estimated from the incidence of all hypoglycaemic episodes occurring during the maintenance period (the treatment period after the initial titration phase which could last up to 6 weeks). Hypoglycaemic episodes were analysed by severity across all trials with an extended Cox regression model including a random effect (following a gamma distribution), which acts multiplicatively on the baseline hazard function and describes the excess risk (or frailty) for a subject. The analysis was adjusted for $HbA_{1c}$ to account for individual differences in glycaemic control during the maintenance period.
**Results:** Very few subjects experienced major hypoglycaemic episodes with either treatment. The risk of experiencing minor hypoglycaemic episodes was significantly lower both nocturnally and over 24 h with insulin detemir compared with NPH insulin. Significantly fewer nocturnal hypoglycaemic episodes registered by symptoms only were reported with insulin detemir than with NPH insulin; however, no treatment difference was detected over 24 h.

| | IDet % (E) | NPH % (E) | IDet/NPH Relative Risk [95% CI] | p-value |
|---|---|---|---|---|
| Nocturnal Episodes | | | | |
| Major | 1.7 (49) | 2.5 (49) | 0.58 [0.30;1.15] | 0.118 |
| Minor | 31.8 (1521) | 37.6 (1371) | 0.65 [0.57;0.76] | 0.000 |
| Symptoms only | 26.1 (1385) | 28.4 (935) | 0.79 [0.66;0.94] | 0.009 |
| Episodes over 24 h | | | | |
| Major | 4.5 (168) | 5.0 (106) | 0.97 [0.61;1.53] | 0.893 |
| Minor | 62.0 (10527) | 62.4 (7283) | 0.86 [0.76;0.96] | 0.010 |
| Symptoms only | 53.5 (9212) | 52.3 (5144) | 0.98 [0.85;1.14] | 0.821 |

% = proportion of subjects with episode(s)
E = episodes

**Conclusion:** The results confirm that treatment with insulin detemir is associated with a lower risk of minor hypoglycaemia. This is a clinically important finding since fear of hypoglycaemia, especially nocturnal hypoglycaemia, is a major obstacle in attaining optimum glucose control and strict fasting glucose targets with intensive insulin therapy.
*Sponsored by: Novo Nordisk*

## 908

**Albumin binding of insulin analogues gives a lower risk for hypoglycaemic events**

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**Background:** Hypoglycaemic events can arise, when variations in absorption rate from a subcutaneous insulin injection leads to variations in the insulin concentration and thereby in insulin action. A novel, basal insulin analogue, insulin detemir, binds reversibly to albumin in plasma and interstitial space. The albumin bound part acts as a buffer that dampens the variations in insulin concentration and stabilises the insulin action. Thus, insulin detemir has in clinical studies demonstrated significantly reduced pharmacokinetic and -dynamic variability and reduced risk of hypoglycaemia, compared to NPH insulin.
**Methods:** We have used a modelling system to describe how a change in subcutaneous absorption rate influences the concentration of insulin detemir or human insulin at the 'receptor compartment', as represented by the interstitial space of the peripheral target tissues.
**Results:** At the injection site, the absorption rate of the albumin bound analogue becomes almost independent of flow velocity, because the flowing albumin buffer acts as a sink that keeps the free concentration in the local capillaries low. This prevents backflow, so the absorption rate mainly depends on the transcapillary permeability. In plasma and interstitial space, albumin binding results in a reduced distribution rate, due to the buffering effect. Plasma half-lives in dogs for human insulin are 7 and 29 min in plasma and interstitial space, respectively, and for insulin detemir 18 and 92 min, respectively. Assuming similar values in humans and using a simple two-compartment system, the effect of a change in absorption rate is calculated.
The results show that a 100% increase in absorption rate at the injection site for periods of 30, 60, or 90 min gives a maximum concentration change of 53%, 80%, or 92% respectively for human insulin, but only 20%, 38%, or 53% respectively for insulin detemir. Variations in absorption rate are thus strongly dampened by albumin binding. For short durations by a factor of 3 and even at 90 min by a factor near 2.
**Conclusion:** The effect of variations in flow velocity is almost eliminated by albumin binding. The effect of other variations in absorption rate is strongly dampened. Albumin binding of insulin is thus an effective tool to reduce variability and provides a new mechanism for reducing the risk of hypoglycaemia.

## 909

**Patients with Type 2 diabetes mellitus have lower rates of nocturnal hypoglycaemia on biphasic insulin aspart (BIAsp30) than on biphasic human insulin-30 (BHI30): data from the REACH study**

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**Background and aims:** Biphasic insulin aspart is a premixed analogue of human insulin (HI) containing 30% soluble and 70% protamine bound insulin aspart. Insulin aspart is a rapid-acting insulin analogue with improved subcutaneous absorption properties compared to HI. Continuous blood glucose monitoring was used to compare the safety aspects of treatment with BHI30 and BIAsp30 in relation to the incidence of hypoglycaemia.
**Materials and methods:** A double-blind, two-period crossover, randomised, multi-centre trial in 160 insulin-treated subjects with type 2 diabetes compared the efficacy and safety of biphasic insulin aspart 30 (BIAsp30) and biphasic human insulin 30 (BHI30). During an eight week run-in period, insulin doses in the currently used regimen were adjusted to achieve pre-breakfast and pre-evening-meal blood glucose levels of 5–7 mmol/l. Patients who achieved an $HbA_{1c}$ 6.5–8.5 % at the end of the run-in period were randomly allocated to treatment with either BIAsp30 or BHI30 twice daily before meals for a 16-week treatment period, then crossed over to the alternative treatment for a further 16 weeks. Insulin total dosage was adjusted using an algorithm in order to improve blood-glucose profiles, based on the targets stated above. The primary assessment variable was the number of glucose readings below 3.5 mmol/l as measured by CGMS (MiniMed) during two 72-hour periods mid-way through and at the end of each treatment period. The frequency of hypoglycaemic episodes (%) was calculated for each study period by dividing the number of blood glucose readings which were less than 3.5 mmol/l by the total number of available readings during the period, and multiplying by 100.
**Results:** Analysis of the intention-to-treat (ITT) population showed fewer BG readings < 3.5 mmol/l measured by CGMS during treatment with BIAsp30 compared to BHI30. The mean frequency of overall hypoglycaemia based on CGMS readings with BIAsp30 compared to BHI30 was 3.80% vs. 4.40%, giving an adjusted mean treatment ratio of 0.85 (p= 0.067). The mean frequency of nocturnal hypoglycaemia was 6.31% for BIAsp30 compared to 7.82% for BHI30 giving an adjusted mean treatment ratio of 0.74 (p=0.020). There was no difference in the mean frequency of reported hypoglycaemic events between treatments, but there were fewer major hypoglycaemic events during treatment with BIAsp30 than with BHI30 (2 patients experienced one episode each with BIAsp 30 and 5 patients experienced a total of 7 episodes when using BHI30). No difference in $HbA_{1c}$ between the two treatments was seen (mean treatment difference BIAsp30 - BHI30 0.06%, p=0.21), and there was no significant difference in patients' satisfaction between the two treatments.
**Conclusion:** Rates of nocturnal hypoglycaemia as measured by CGMS in this population of type 2 diabetes patients previously treated with insulin were significantly lower when using BIAsp30 than when using BHI30, with no difference in overall control.
*Supported by: Novo Nordisk Ltd.*

## 910

**The AT.LANTUS trial investigating treatment algorithms for insulin glargine therapy: results in patients with Type 1 and Type 2 diabetes**

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**Background and aims:** Numerous studies have demonstrated the importance of achieving and maintaining tight metabolic control to prevent diabetic complications. However, achieving normal blood glucose control is limited by insufficient insulin titration due to fear of hypoglycaemia, weight gain and complicated treatment regimens. The primary aim of this 24-week, multicentre (1012 centres), multinational (59 countries), randomized study was to investigate the most effective way of using insulin glargine in poorly controlled Type 1 and Type 2 subjects to overcome these obstacles. Two algorithms (Algs) were compared in terms of severe hypoglycaemia (assistance with BG <2.8 mmol/L [<50 mg/dL]).
**Materials and methods:** 7371 subjects were randomized to Alg1 (Type 1, n=1172; Type 2, n=2493) or Alg2 (Type 1, n=1238 Type 2, n=2468). For Type 1 study, Alg1 was a fixed dose titration and Alg2 was a variable dose titration. For Type 2, Alg1 was a visit-based titration using 2–8 IU increments (10 IU start dose for insulin-naïve patients). Alg2 titrated 2 IU every 3 days with patient self-titration (first dose based on FBG for insulin-naïve patients).
**Results:** 2410 patients with Type 1 diabetes (55.5% female, median age 35.8 years, mean $HbA_{1c}$ 8.5 ± 1.2%, mean body mass index [BMI] 24.7 kg/m$^2$; mean diabetes duration 14.7 ± 10.3 years) and 4961 patients with Type 2 diabetes (52% female, median age 57.6 years, mean $HbA_{1c}$ 8.9 ± 1.3%, mean BMI 29.0 kg/m$^2$, mean diabetes duration 12.3 ± 7.2 years) were changed from their previous treatment to an insulin glargine based regimen. There was no significant difference in severe hypoglycaemia/100 patient-years between Alg1 or Alg2 for patients with Type 1 (16.5 vs 14.3) or Type 2 (1.9 vs 2.4) diabetes. There was a significant decrease in $HbA_{1c}$ and FBG (p <0.001) in Type 1 and Type 2 patients on both sets of Algs; furthermore, there were clinically relevant statistical differences (p< 0.001) between the treatment algorithms in the Type 2 study in which the subjects were involved in the self-management of basal insulin titration. There was a slight increase in body weight in both studies (see table).
**Conclusions:** This study shows that insulin glargine is effective in improving metabolic control in subjects at an advanced stage of their disease with no differences between the two sets of Algs in the incidence of severe hypoglycaemia. The results demonstrate that insulin glargine can be safely used by a diverse population of advanced and poorly controlled patients with Type 1 or Type 2 diabetes to optimize glycaemic control. Significant differences in the additional reductions of $HbA_{1c}$ and FBG were, however, found in the Type 2 study with Alg2.

| | | Algorithm 1 | | Algorithm 2 | |
|---|---|---|---|---|---|
| | | Baseline | Endpoint | Baseline | Endpoint |
| Insulin glargine dose (IU/day) | Type 1 | 24.2 ± 11.8 | 29.9 ± 14.8 | 23.7 ± 11.3 | 29.6 ± 13.5 |
| | Type 2 | 22.3 ± 15.1 | 41.0 ± 22.8 | 23.5 ± 15.8 | 45.0 ± 27.8 |
| $HbA_{1c}$ (%) | Type 1 | 8.5 ± 1.2 | 7.8 ± 1.2 | 8.6 ± 1.2 | 7.8 ± 1.2 |
| | Type 2 | 8.9 ± 1.3 | 7.9 ± 1.2 | 8.9 ± 1.3 | 7.7 ± 1.2 (alg1 vs alg2; p<0.001) |
| Fasting blood glucose, mmol/L (mg/dL) | Type 1 | 9.9 ± 3.6 (178.5 ± 64.8) | 6.7 ± 2.2 (121.1 ± 40.0) | 9.9 ± 3.4 (178.1 ± 61.2) | 6.6 ± 2.3 (118.8 ± 41.2) |
| | Type 2 | 9.4 ± 2.8 (169.8 ± 49.9) | 6.3 ± 1.7 (112.9 ± 31.4) | 9.4 ± 2.8 (169.4 ± 50.8) | 6.0 ± 1.6 (107.7 ± 28.7) (alg1 vs alg2; p<0.001) |
| Body weight (kg) | Type 1 | 70.7 ± 13.8 | 71.3 ± 14.3 | 69.9 ± 13.1 | 70.5 ± 13.4 |
| | Type 2 | 79.7 ± 15.8 | 80.8 ± 16.0 | 79.8 ± 16.2 | 81.1 ± 16.5 |

*Supported by: Aventis Pharma.*

## 911

**The AT. LANTUS trial investigating treatment algorithms for insulin glargine therapy: results of the Type 1 study**

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**Background and aims:** Insulin glargine (LANTUS®), a once-daily basal insulin analogue, has been shown to achieve better metabolic control than NPH insulin in patients with Type 1 diabetes, without an increased risk of hypoglycaemia. The primary aim of this 24-week, open-label, randomized, multinational (409 centres, 57 countries) study was to investigate the optimal method for initiating and maintaining insulin glargine therapy in patients with Type 1 diabetes by comparing two algorithms (Algs) in terms of severe hypoglycaemia (equivalence range = 4% to 4%).
**Materials and methods:** 2416 patients (55.5% female, median age 35.8 years, mean diabetes duration 14.7 ± 10.3 years) were randomized to Alg1: fixed dose titration (based on a 10% dose increase, without exceeding 4 IU) or Alg2: variable dose titration (2–6 IU dose increments according to FBG value). The titration was based on a target FBG of 4.4–6.7 mmol/L (80–120 mg/dL). Mean basal insulin dose was 0.43 ± 0.21 IU/kg. Mean $HbA_{1c}$ at baseline was 8.47 ± 1.16% (Alg1) and 8.55 ± 1.15% (Alg2), mean fasting blood glucose (FBG) was 9.9 ± 3.6 mmol/L (178.5 ± 64.8 mg/dL; Alg1) and 9.9 ± 3.4 mmol/L (178.5 ± 61.2 mg/dL; Alg2). The mean starting dose of insulin glargine was 0.35 ± 0.16 IU/kg (Alg1) and 0.34 ± 0.16 IU/kg (Alg2).
**Results:** There was no significant difference in the incidence of severe hypoglycaemia between the two Algs (7.7 vs 6.8% [Alg1 vs Alg2]; 90% confidence interval [CI] [-2.8 to 0.9%]). There was no significant difference between the two Algs in terms of nocturnal (22.6 vs 22.1% [Alg1 vs Alg2]) or symptomatic hypoglycaemia (79.7 vs 80.1% [Alg1 vs Alg2]). Basal insulin doses increased in both Algs (0.41 ± 0.17 IU/kg [Alg1]; 0.42 ± 0.17 IU/kg [Alg2]) p <0.001). There was a significant decrease in FBG in both Algs (3.2 ± 3.8 mmol/L [57.4 ± 69.0 mg/dL] vs 3.3 ± 3.7 mmol/L [59.4 ± 67.3 mg/dL; Alg1 vs Alg2]; p <0.001). Similarly, $HbA_{1c}$ significantly decreased in both Algs (0.64 ± 1.21% [Alg1]; 0.72 ± 1.21% [Alg2]; p <0.001).
**Conclusion:** This study shows that both Algs are effective in improving metabolic control in patients with Type 1 diabetes, with no clinically significant differences in terms of efficacy or incidence of severe hypoglycaemia, and can be safely used to optimize glycaemic control in patients with Type 1 diabetes.
*Supported by: Aventis Pharma.*

## 912

**Insulin glargine is associated with less hypoglycaemia than NPH insulin in older patients with Type 2 diabetes**

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**Background and aims:** Hypoglycaemia is feared as a possible consequence of insulin treatment, especially in older patients who may be vulnerable to falls and fractures. Insulin glargine (LANTUS®; GLAR) has previously been shown to cause less hypoglycaemia than NPH insulin (NPH) in various populations, but this benefit has not been carefully demonstrated in patients over the age of 65 years.
**Methods and materials:** We further analysed data from four randomized trials in which patients with Type 2 diabetes were treated with GLAR once daily or NPH once- or twice-daily for 20–28 weeks using a structured insulin protocol. In three trials, oral agents were continued when insulin glargine or NPH were initiated; in one trial, insulin was previously used and prior mealtime insulin injections were continued.
**Results:** Of 2304 patients in the original intent-to-treat populations, 660 were ≥65 years old. They had a mean body mass index of 29.2 kg/m² and a duration of diabetes of 13.3 years; 592 were aged 65–75 years and 68 were ≥75 years old. Mean $HbA_{1c}$ at baseline (GLAR vs NPH-treated patients) was 8.62 vs 8.57% (65–75 years) and 8.93 vs 8.53% (≥75 years). Mean $HbA_{1c}$ at endpoint was 7.75 vs 7.76% (65–75 years) and 8.17 vs 8.05% (≥75 years). GLAR and NPH were associated with similar baseline-adjusted improvements in $HbA_{1c}$ in both age groups: -0.87 vs -0.82% (65–75 years); -0.76 vs -0.48% (≥75 years), GLAR versus NPH. Mean daily basal insulin dose at endpoint (GLAR versus NPH) was 29.9 vs 31.4 IU (65–75 years) and 25.1 versus 24.8 IU (≥75 years). The use of oral antidiabetic drugs (OADs) was similar between treatments and across age groups. Rates of hypoglycaemia are shown in the table below.

| | Age 65–75 years | | | | | Age ≥75 years | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Hypoglycaemia | GLAR (n=285) | | NPH (n=307) | | % risk reduction with GLAR† | GLAR (n=36) | | NPH (n=32) | | % risk reduction with GLAR† |
| | n | % | n | % | | n | % | n | % | |
| All symptomatic | 148 | 52 | 178 | 58 | 10% | 11 | 31* | 16 | 50 | 39% |
| Confirmed ≤3.1 mmol/L (≤56 mg/dL) | 76 | 27* | 110 | 36 | 26% | 6 | 17 | 12 | 38 | 56% |
| Nocturnal | 73 | 26* | 114 | 37 | 31% | 5 | 14 | 10 | 31 | 56% |
| Severe (requiring assistance) | 4 | 1 | 11 | 4 | 61% | 0 | 0 | 0 | 0 | 0% |

*p <0.05 versus NPH insulin; NPH insulin=denominator

GLAR-treated patients were significantly less likely to have confirmed hypoglycaemia ≤3.1 mmol/L (≤56 mg/dL) (-26%) and nocturnal hypoglycaemia (-31%) than those taking NPH in the 65–75 year age group. Although there were modest numbers of patients in the ≥75 year age group, significantly fewer of them (-39%) had symptomatic events with insulin glargine compared with NPH insulin.
**Conclusion:** At equivalent levels of glycaemic control by $HbA_{1c}$, GLAR was less likely than NPH to cause hypoglycaemia. Thus, in older patients as in the general population of patients with Type 2 diabetes, GLAR is a safer alternative to NPH insulin.
*Supported by: Aventis Pharma.*

## 913

**Insulin detemir is associated with lower risk of hypoglycemia compared to NPH insulin in people with Type 1 diabetes**

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**Background and aims:** Hypoglycaemia remains a major barrier to achieving optimal glycaemic control in people with Type 1 diabetes mainly due to the un-physiological properties of traditional insulin preparations. We investigated if basal-bolus therapy with insulin detemir, a new soluble basal insulin analogue, was superior to NPH insulin (NPH) in reducing the risk of hypoglycaemia.
**Materials and methods:** In this multinational, open-label trial, 70 men and 60 women with Type 1 diabetes received insulin detemir and NPH insulin twice daily (morning and bedtime) in a randomised cross-over design in combination with pre-meal insulin aspart during two 16-week treatment periods. Baseline $HbA_{1c}$: 7.9 ± 0.7%, (mean±SD), age: 39.2 ± 12.3 yrs, diabetes duration 16.6 ± 10.2 yrs, and BMI: 25.3 ± 3.5 kg/m$^2$. Home-measured plasma glucose was recorded on 4 days during the last week of each treatment period. A subset of subjects was admitted to the hospital overnight during the last week of each treatment period and plasma glucose was sampled every 30 min. Hypoglycaemia was analysed during the last 10 weeks of each treatment period and classified as *major* (assistance needed), *minor* (plasma glucose < 3.1 mmol/L, no assistance needed), *symptoms only* (plasma glucose not measured or ≥ 3.1 mmol/L).
**Results:** Risk of nocturnal (23:00–06.00) as well as overall hypoglycaemia was 50% [95% CI: 0.38; 0.65] and 18%, [95% CI: 0.73; 0.92] lower with insulin detemir than with NPH insulin, respectively. Incidence of major hypoglycaemic episodes was numerically lower with insulin detemir: relative risk (insulin detemir/NPH): 0.61, [95% CI: 0.27; 1.36]. $HbA_{1c}$ decreased by 0.3% point with both treatments and was comparable at 7.6% [95% CI: –0.11; 0.11] after 16 weeks. Within-person variation in mean home-measured plasma glucose was lower with insulin detemir than with NPH insulin (SD: 3.0 vs 3.3 mmol/L, p<0.001) as was home-measured fasting plasma glucose, (insulin detemir: 7.63, NPH: 8.66 mmol/L, p<0.0001). Nocturnal plasma glucose excursions < 4 mmol/L, measured for 43 persons in hospital were also lower with insulin detemir (p=0.028). The general safety profiles of insulin detemir and NPH insulin were comparable.
**Conclusion:** Insulin detemir was associated with a significantly lower risk of hypoglycaemia compared to NPH insulin at similar $HbA_{1c}$ when used in a basal-bolus regimen. The lower risk of hypoglycaemia is probably related to the more stable and predictable plasma glucose levels observed with insulin detemir compared to NPH insulin and thus allow people to achieve tighter glycaemic control.
*Sponsored by: Novo Nordisk*

# PS 85

# Diabetes and other endocrinopathies/co-morbidities

## 914

**The peculiarities of thyroid gland characteristics in patients with metabolic syndrome**

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**Background and aims:** Metabolic syndrome (MS) is most common problem of overweight population. During the last 20 years the incidence of hypothyreosis is also frequently found in overweight population. The aim of our study was the assessment of the possible relationships between MS and structural and functional abnormalities of thyroid gland.
**Materials and methods:** We have studied 51 overweight patients (36 females, age – 45.8 ± 10.3; BMI – 32.7 ± 4.9; 15 males, age – 51.4 ± 11.5; BMI – 32.8 ± 3.2). Patients were selected by clinical definitions of World Health Organisation (WHO) and National Cholesterol Education Program Adult Treatment Panel III (ATP III). By WHO definition MS was diagnosed in 38 patients (MS-WHO group), and by ATP III definition - in 45 patients (MS-ATPIII group). Control group consisted of non-MS-patients. BMI, waist circumference (WC), WHR, and fasting plasma glucose (FPG), postprandial plasma glucose (PPG), serum basal C-peptide (BCP), serum triglycerides (Tg), serum HDL-cholesterol (HDL), BP levels have been investigated; TG volume (TGV) and structure has been studied by ultrasonography; function of TG has been evaluated by determination of serum TSH and FT4 levels.
**Results:** The obtained results showed that TGV correlated with age (r = –0.516, p=0.002), duration of diabetes mellitus type 2 (r = –0.381, p=0.024), Tg (r = –0.568, p=0.002), systolic BP (r = –0.487, p=0.008); Correlation with other parameters of MS features did not take place. It is necessary to note, that characteristics of TG function were not correlated with all parameters of MS features. The comparison of characteristics of MS-WHO group with control group showed that significant difference takes place between Ages (49.3 ± 10.7 vs. 42.1 ± 9.6, p=0.032), WC (107.4 ± 10.7 vs. 96.1 ± 9.0 cm, p < 0.001), FPG (151.7 ± 71.8 vs. 91.5 ± 18.0 mg/dl, p<0.001), PPG (180.3 ± 80.9 vs. 104.9 ± 32.4 mg/dl, p<0.001), BCP (4.4 ± 1.3 vs. 3.2 ± 1.4 ng/dl, p=0.028), Tg (220.4 ± 131.3 vs. 99.3 ± 31.1 mg/dl, p<0.001); the significant difference in the characteristics of TG did not occur. The comparison of characteristics of MS-ATPIII group with control group showed that significant difference takes place between WC (105.9 ± 10.2 vs. 93.5 ± 9.9 cm, p=0.026), FPG (143.3 ± 71.2 vs. 101.7 ± 18.5 mg/dl, p=0.006), Tg (204.1 ± 130.4 vs. 105.8 ± 24.4 mg/dl, p<0.001), HDL (41.6 ± 9.3 vs. 59.6 ± 11.7 mg/dl, p=0.024); TSH (2.5 ± 3.6 vs. 0.8 ± 0.4 μU/ml, p=0.008).
**Conclusion:** Our results suggests that thyroid pathology manifested as hypothyreosis is related with the MS and characteristics of its features. This suggestion has to be documented by further investigations.

## 915

**Subclinical thyroid disease in Type 2 diabetes. Is it worth to screen?**

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**Background and aims:** Subclinical hypothyroidism and hyperthyroidism are diagnoses based on laboratory thyroid function evaluation with minimal clinical signs or symptoms. There is still great uncertainty concerning the consequences of not treating versus the benefit of treatment. The screening for thyroid dysfunction is accepted for type 1 diabetes in the context of auto-immunity. Its role for type 2 diabetes is controversial. Thyroid dysfunction can be associated with alterations in lipid parameters that are already altered in this population. We analysed the prevalence of subclinical thyroid dysfunction in a population of type 2 diabetic patients and correlated this finding with lipid parameters.
**Materials and methods:** Clinical files of patients admitted (in 2002–2003) for the first time in our clinic were analysed. 2204 type 2 diabetics were found: age- 60.6 +/–0.22 years; years of evolution-10.1 +/–0.19 years.
The levels of TSH, Hb A1 c, total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol were measured. Subclinical hypothyroidism was defined if the values of TSH were between 4.5–10 uUI/mL and subclinical hyperthyroidism if these values were between 0.15–0.45 uUI/mL. A non-parametric analysis (Kruskall-Wallis) was performed between these variables.

**Results:** Undiagnosed subclinical hyperthyroidism was found in 2.8% and subclinical hypothyroidism in 3.4% of our population. Hypothyroidism was diagnosed in 64% of cases between 50 and 70 years old (and during the first 10 years of diagnosis).
A positive association was found between total cholesterol and thyroid function (subclinical hypothyroidism: 243 mg/dl+/–5.31 vs euthyroidism: 228 mg/dL+/–1.11; p=0.038). No association was found between thyroid dysfunction and others variables evaluated. By contrast, a strong association was found between HbA1 c and triglycerides (p=0.005), HDL-cholesterol (p=0.02) and total cholesterol (p=0.001).
**Conclusion:** We found 6.2% of type 2 diabetic patients with undiagnosed subclinical hypo and hyperthyroidism. There was an association between thyroid function and total cholesterol. However, as expected, there was a stronger correlation between lipid parameters and the degree of metabolic compensation.
Type 2 diabetes is associated with lipid alterations. Current guidelines advise an early therapeutic intervention correcting the existing dyslipemia. With this work we present another cause of possible dislipidemia that may coexist in a type 2 diabetic patient. A therapeutic intervention restoring a normal thyroid function may ameliorate these lipid abnormalities.

## 916

**Coeliac disease in Type 1 diabetic patients: a long-term prospective investigation**

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**Background and aims:** Type 1 diabetes (T1D) is often associated with other autoimmune disorders, including Coeliac Disease (CD). Subclinical CD is usually detected at the time of T1D diagnosis, but some evidence indicates that CD-related antibodies (CD-A) can also appear later, even several years after the onset of T1D. This implies that T1D patients must be screened for CD-A regularly over time, in order to detect early signs of CD. Thus the aim of the study was to evaluate the frequency of CD-A in a large cohort of newly paediatric T1D patients at diagnosis and quantify the first appearance of CD-A after that.
**Materials and methods:** The study started in 1991 and ended in December 2002. During this time, 381 newly diagnosed T1D patients [M:F=185:196; median age (range) at diagnosis: 8.4 years (0.7–17.7)] have been prospectively monitored for a median period of 6.5 years (range: 2.7–11.2). At the time of T1D onset, screening for CD included an evaluation of EMA and levels of IgG, IgA and IgM. If the patient was IgA deficient, IgG-AGA were measured. Except for IgG, IgA and IgM, EMA and/or IgG-AGA determinations were repeated every 6–12 months. Patients who resulted positive for EMA or IgG-AGA in two consecutive occasions underwent intestinal biopsy.
**Results:** Four patients (1.0%) were diagnosed with CD before the onset of T1D and were therefore excluded from the study. Of the remaining 377 newly diagnosed T1D patients, 18 (4.8%) were found positive for CD-A (17 for EMA e 1 for IgG-AGA). Intestinal biopsy was obtained from 17 patients (one patient positive for EMA refused it) and in 16 the diagnosis of CD was confirmed histologically. The median age of T1D onset in these 16 patients was 9.0 years (range: 1.5–12.3). Therefore, the prevalence of CD at the time of T1D diagnosis in out cohort was 4.2%. Of the 359 patients, who were negative for CD-A at the time of onset of T1D, 8 (2.2%) seroconverted to EMA positivity; the interval of the seroconversion varied from 1.5 to 7.5 years from the diagnosis of T1D. Intestinal biopsy was obtained in all the 8 patients and CD was confirmed histologically in 7 of them. Therefore, the overall rate of progression to CD in our T1D cohort was 2.0%. At the time of diagnosis of T1D, the 7 patients had an age between 0.7 to 6.9 years. By taking 8.4 years as the median age at onset of T1D of the whole cohort in our study and applying it to the Kaplan-Meier analysis, patients in the younger group had an increased risk of developing CD compared to those who developed T1D at an older age (7/185 vs 0/183, respectively; log rank test p=0.0086).
**Conclusion:** Our study confirms that screening of CD-A is an useful tool for identifying subclinical CD among patients with T1D. In our cohort, the prevalence of subclinical CD in newly diagnosed T1D patients was 4.2% and increased up to 6.2% during the period of the follow-up. Patients who developed T1D at a younger age had a higher risk of developing CD over time. The recommendations which emerge from our study are: 1) all children who develop T1D must be screened for CD-A at diagnosis, regardless of the age of onset of the disease; 2) children who develop T1D within the first 9–10 years of age have to be screened to CD-A at a close follow-up (every 6–12 months), while in the ones who develop T1D at an older age, the screening for CD-A should be done at a more prolonged interval of time (3–5 years).

## 917

**Does Type 2 diabetes influence the bone mineral density of women after surgically induced menopause?**

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**Background and aims:** Surgically induced menopause resulting in abrupt estrogen cessation is an established risk factor for osteoporosis while the influence of type 2 diabetes on bone metabolism is still controversial. In this study we investigated the effect of type 2 diabetes on the bone mineral density (BMD) of women who underwent bilateral oophorectomy.
**Materials and methods:** The BMD of L2-L4 vertebrae and femoral neck (FN) was measured in 16 diabetic women [group D-SUMP, age 52.1 ± 5.8 years (x ± 1 SD), age at menopause 43.8 ± 0.7, years since menopause 8.2 ± 5.2, BMI 29 ± 2.9 kg/m$^2$, diabetes duration 6.5 ± 3.9 years, HbA1 c levels 6.2 ± 0.5%] and 97 non diabetic women (group SUMP, 53.4 ± 5.3, 41.4 ± 2.6, 10.1 ± 6, 26.8 ± 3.6 respectively). All women underwent bilateral oophorectomy at an age less than 46 years and didn't suffer from any other disease with known influence on bone metabolism. In diabetic women the diabetes duration always exerted the 75% of time elapsed after surgery.
**Results:** The absolute BMD values as well as the respective age-matched ones (Z scores) of L2-L4 vertebrae were significantly higher in the D-SUMP group compared to the SUMP one (0.960 ± 0.124 gr/cm$^2$ and –0.23 ± 0.93 vs 0.876 ± 0.142 gr/cm$^2$ and –0.87 ± 1.12 respectively, p<0.05). No significant difference existed between the 2 groups regarding BMD values of FN. The proportions of osteoporotic-osteopenic (OPO-OPE) women based on vertebral T score values were significantly higher as a whole in SUMP compared to D-SUMP women (OPO=24% and OPE=48% vs 8% and 46% respectively, p<0.05). The same proportions based on FN T score values didn't differ significantly between SUMP and D-SUMP (OPO= 23% vs 25% and OPE=50% vs 44%). In D-SUMP there was a significant positive correlation between BMI and the vertebral (r=0.64, p<0.05) but not the FN (r=–0.01) BMD values. In SUMP women both vertebral and FN BMD values were positively correlated to BMI (r=0.22 and 0.21 respectively, p<0.05). In D-SUMP no significant correlation was observed between BMD values and either diabetes duration or HbA1 c levels.
**Conclusion:** Type 2 diabetes seems to favour the BMD of cancellous bone in women who underwent bilateral oophorectomy in a relatively young age. Neither the disease duration nor the degree of glucose control seem to influence the bone density in that group of women.

## 918

**Moderate increase in protein intake does not normalize the IGF-system in patients with Type 1 diabetes**

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**Background and aims:** The IGF-system is disturbed in type 1 diabetes. These changes, which might be of importance for development of diabetes complications, include low IGF-I, high GH and changes in the levels of binding proteins for IGF-I. Our aim was to study if these changes, as supported by experimental data in animals, can be affected by dietary protein intake.
**Materials and methods:** Twelve patients with type 1 diabetes, age 37.5 ± 10.0 years, diabetes duration 20.1 ± 9.3 years and HbA1 c 6.3 ± 0.6 % were randomized to a diet with normal protein content (10E%; 0.9 g protein/kg body weight) or to a diet with higher protein content (20 E%; 1.8 g protein/kg body weight) in a randomized cross-over study. The treatment periods were 10 days with a wash-out period of 11 days. A control group matched for age and sex only underwent the basal testing. Total and free IGF-I and -II, IGFBP-1, -2 and -3, GH and GH binding protein as well as ghrelin were measured with validated immunoassays.
**Results:** The urinary excretion of urea was 654 ± 159 mmol/24 h at day 10 of the high protein diet and 320 ± 75 mmol/24 h at day 10 of the normal protein diet (p<0.001). There were no changes in body weight or glycaemic control between the diets. Overnight fasting levels of IGF-I were 117 ± 28 µg/L after high protein diet and 121 ± 33 µg/L after normal protein diet (ns)

and the corresponding concentrations of IGFBP-1 were 15 ± 11 and 13 ± 13 µg/L (ns). There were no differences in plasma concentrations of free IGF-I, free IGF-2, IGFBP-3, GHBP and ghrelin but a small difference was found in IGFBP-2 (263 ± 66 vs. 302 ± 97 µg/L; low vs. high protein; $p<0.04$). Compared to the patients the control group had lower total IGF-I and higher IGFBP-1.
**Conclusion:** A moderate change of the dietary protein intake of patients with type 1 diabetes does not influence IGF-I. In order to normalize the IGF-system other interventions must be used.

## 919

**Effect of apo E4 allele on plasma LDL-cholesterol response to calorie restricted diet therapy in Type 2 diabetic patients**
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**Background and aims:** Apo E4 is one of the candidate genes which may contribute to responsiveness to diet therapy, but it remains controversial. In diabetic patients there is little information concerning the relationship between apo E4 allele and plasma LDL-cholesterol response to diet therapy. Our previous study showed that hypercholesterolemia in type 2 diabetic patients with apo E4 allele is more closely related to poor glycemic control. The aim of this study is to investigate the effect of apo E4 allele on plasma LDL-cholesterol response to calorie restricted diet therapy in type 2 diabetic patients.
**Materials and methods:** Twenty four diabetic patients with apo E3/3 genotype and 11 diabetic patients with apo E4/3 genotype were recruited. They were hospitalized for calorie restricted diet therapy (25.0 kcal/kg body weight/day) for 14 days. Body weight, fasting plasma glucose (FPG) levels and plasma lipid levels on hospital days 1 and 14 were compared between the two apo E genotype groups.
**Results:** There were no significant differences in baseline FPG levels, HbA1 c levels, BMI, plasma levels of total cholesterol, triglyceride and HDL-cholesterol between the two apo E genotype groups, but baseline plasma levels of LDL-cholesterol were significantly higher in apo E4/3 group (147 mg/dl) than in apo E3/3 group (134 mg/dl). Body weight decreased slightly, and FPG levels decreased significantly after diet therapy in both apo E genotype groups. In apo E3/3 group only plasma levels of triglyceride decreased significantly ($p<0.05$) after diet therapy, whereas in apo E4/3 group plasma levels of triglyceride, total cholesterol and LDL-cholesterol decreased significantly ($p<0.05$, $p<0.001$ and $p<0.05$, respectively) after diet therapy. The decrease (percent change) in total cholesterol (−16.3% vs −6.6%) and LDL-cholesterol (−15.6% vs −0.7%) after diet therapy was significantly ($p<0.001$) greater in apo E4/3 group than in apo E3/3 group.
**Conclusion:** The 14-day calorie restricted diet therapy is a beneficial measure to treat hypercholesterolemia in type 2 diabetic patients with apo E4/3 genotype since apo E4 allele appears to link calorie restriction to greater improvement of plasma LDL-cholesterol.

# PS 86
# Quality of life and cognitive function

## 920

**Determinants of treatment satisfaction in insulin treated patients with diabetes mellitus**
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**Background and aims:** Up to the present there is large discussion about most important determinants (i.e. quality of diabetes control, treatment strategy, presence of long-term complications) of treatment satisfaction (TS) in patients with insulin treated diabetes mellitus (DM). Hence, it was the aim of the trial to assess TS population-based, but also in specific cohorts of patients with insulin treated DM.
**Materials and methods:** Totally 338 patients (type 1/2 n=191/147) were included in the trial. TS was assessed using a standardised questionnaire according to Bradley et al. Additionally in all patients quality of diabetes control (HbA1 c, Tosho, mean normal 5,05%), treatment strategy (insulin injections, frequency of insulin dose adaption, self-monitoring, diet) and long-term complications were studied. Analyses were performed in 4 cohorts: 1) 90% of all the patients aged 16–60 ys and living in the city of Jena, Germany (type 1/2 n=113/98) (population-based survey), 2) all the patients with type 1 DM (n=45) participated in a structured treatment and teaching programme for intensified insulin therapy during a 12 months period, 3) all the patients with type 1 DM (n=33) treated with insulin pumps during the same period at our center, and 4) 49 patients with type 2 DM, participated in a randomised trial comparing therapy with a long-acting insulin analog (Glargine) with conventional insulin therapy.
**Results:** Neither in the total cohort, nor in subgroups there were correlations or associations (multivariate analysis) between TS and the quality of diabetes control or treatment strategy. Population-based patients with type 1 DM (age 40.4 ± 12.2 ys, HbA1 c 8.4 ± 1.8%) and peripheral polyneuropathy had lower TS (Table). In patients with type 2 DM (age 53.2 ± 6.4 ys, HbA1 c 9.1 ± 1.9%) there were no differences. Additionally there were no differences between patients with type 2 DM and conventional (n=65, TS 33.6 ± 8.7 pts) or intensified forms of insulin therapy (n=33, TS 33.6 ± 7.8 pts, p=0.75). Patients with type 1 DM and insulin pumps (n=33, age 36.7 ± 11.7 ys, HbA1 c 7.4 ± 1.08%, TS 32.8 ± 5.9 pts) had better TS compared with matched-pairs using intensified insulin therapy without pumps (TS 29.8 ± 5.2 pts, p=0.04).
**Conclusion:** In patients with type 1 and type 2 DM TS is not associated with the quality of diabetes control. Patients with type 1 DM and insulin pump therapy have higher TS. Population-based in type 1 DM the most important parameter reducing TS is peripheral polyneuropathy. In type 2 DM there are no differences in TS between patients using conventional or intensified insulin treatment strategies or a long-acting insulin analog.

Table. Treatment satisfaction assessed population-based in patients with type 1 and 2 diabetes.

| Type 1 (n=98) | With | Without | p-value |
|---|---|---|---|
| Neuropathy | 30.6 ± 7.8 (n=28) | 33.0 ± 6.0 (n=85) | 0.08 |
| Nephropathy | 31.4 ± 5.9 (n=28) | 32.9 ± 6.9 (n=79) | 0.32 |
| Retinopathy | 31.8 ± 6.2 (n=56) | 32.8 ± 7.7 (n=37) | 0.48 |
| **Type 2 (n=113)** | | | |
| Neuropathy | 31.3 ± 9.1 (n=40) | 34.4 ± 8.6 (n=58) | 0.09 |
| Nephropathy | 33.9 ± 10.1 (n=40) | 32.7 ± 8.3 (n=55) | 0.54 |
| Retinopathy | 35.5 ± 10.1 (n=31) | 32.3 ± 8.0 (n=41) | 0.14 |

## 921

**Association between psychological general well-being and both glycemic control and dyslipidemia in patients with Type 2 diabetes**
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**Background and aims:** Well-being is an important aspect of the quality of life of patients with type 2 diabetes. The aim of this study was to determine

the extent to which psychological well-being is associated with glycemic control, diabetic dyslipidemia, diabetes-related symptoms, and demographics,
**Materials and methods:** We administered the Psychological General Well-Being Schedule (PGWB) and the Hyperglycemia, Hypoglycemia, Fatigue, and Cognitive Distress subscales of the Diabetes Symptom Checklist-Revised (DSC-R) to 151 patients with type 2 diabetes and diabetic dyslipidemia. These patients were recruited for a clinical trial of oral anti-diabetes treatment (62% male, Means: age = 55 years, BMI = 31, A1C = 8.4%, LDL = 118 mg/dL, HDL = 41 mg/dL, triglycerides = 250 mg/dL). The PGWB consists of 22 items divided into six subscales: anxiety, depressed mood, positive well-being, self-control, general health, and vitality. We conducted seven stepwise multiple regression analyses using the six subscale scores and the total score as dependent variables. A1C, LDL-C, HDL-C, triglycerides (TG), age, gender, BMI, and self-reported hyperglycemia, hypoglycemia, fatigue, and cognitive distress symptoms were used as independent variables.
**Results:** Intercorrelations between variables in different categories of dependent variables (i.e., lab values, demographics, symptoms) were less than 0.25 with the exception of r = 0.30 ($p < 0.05$) between A1C and hyperglycemia symptoms. Total R squared (variance accounted for) and significant predictors ($p < 0.05$), listed in the order in which they were entered into the model, are listed in Table 1 by dependent variable. Fatigue was a significant predictor of every aspect of psychological well-being except self-control and hypoglycemia was a significant predictor of every aspect but vitality. In general, neither lab values nor demographics predicted well-being.
**Conclusion:** The results suggest that the well-being of patients with type 2 diabetes is largely dependent on their symptom severity and not the level of their risk factors for microvascular and macrovascular complications.

Results of Seven Multiple Regression Analyses

| Dependent Variable | Predictors | R squared |
|---|---|---|
| Depression | Hypoglycemia, fatigue, TGs | 0.43 |
| General health | Fatigue, hyperglycemia, hypoglycemia | 0.44 |
| Positive well-being | Hypoglycemia, fatigue | 0.41 |
| Anxiety | Hypoglycemia, fatigue | 0.42 |
| Self-control | Hypoglycemia, cognitive distress | 0.48 |
| Vitality | Fatigue, age, BMI | 0.65 |
| Total PGWB | Fatigue, hypoglycemia | 0.60 |

*Supported by: Eli Lilly & Company*

## 922

**Concerns about insulin therapy in Type 2 diabetic patients**
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**Background and aims:** Concerns about insulin therapy (IT) could be a barrier for type 2 diabetic patients to initiate and perform an IT properly. The aim of this study was to compare concerns about IT in type 2 diabetic patients, in which the initiation of an IT is planned, to type 2 diabetic patients who are already treated with insulin. Additionally these two patients groups will be compared to each other with regard to symptoms of depression, anxiety and to emotional problems associated with diabetes.
**Materials and methods:** 126 type 2 diabetic patients (HbA1 c 8.6 ± 1.7%, age: 60.3 ± 10.6 yrs.; disease duration: 12.7 ± 8.5 yrs.; gender: 44% female) completed an anxiety questionnaire (State Trait Anxiety Inventory), a depression inventory (Hamilton Depression Scale) and the Problem Areas in Diabetes questionnaire (PAID). Also these patients completed a new developed questionnaire designed to measure concerns about IT (CIT). This questionnaire consists of three scales: „barriers for IT" ($\alpha$=.91), „fears against certain aspects of IT" ($\alpha$=.95) and „emotional impact of IT" ($\alpha$=.93).
**Results:** Patients, in which initiation of IT is planned (n=39), reported significantly more barriers for IT (12.3 ± 5.5 vs. 7.0 ± 4.6; P<.01), greater fears against IT (9.5 ± 6.1 vs. 4.2 ± 3.6 P<.01), a greater negative emotional impact of IT (8.3 ± 8.7 vs. 3.5 ± 2.8; P <.01) and more problem areas in diabetes ( 30.1 ± 17.4 vs. 21.7 ± 14.7; P<.01) than patients, who are already transferred to an IT (n=87). No significant differences in depression and trait anxiety were observed. Problem areas in diabetes were highly correlated to the subscales of the CIT („barriers for IT" r=.60; „fears against IT" r=.62; „emotional impact of IT" r=.36).
**Conclusion:** Perceived barriers for IT, fears against IT as well as negative emotional impact of IT seems to be greater before the initiation of IT. These concerns should be addressed in diabetic education programs for these patients, because these concerns seems to be associated with more diabetes related emotional problems in general. This cross sectional study may also indicate that concerns about IT may decline remarkably after accommodation to this therapy form. There is a great need for longitudinal studies on this matter.

## 923

**Validation of a diabetes-specific quality of life questionnaire measure (ADDQoL) for the Russian diabetic population**
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**Background and aims:** Until now, there was no validated instrument to measure diabetes-specific quality of life in the Russian-speaking patient population. We aimed to perform linguistic and psychometric validation of the Audit of Diabetes Dependent Quality of Life (ADDQoL) questionnaire, which is an individualised tool with proven reliability, validity and responsiveness in the English-speaking diabetic population.
**Materials and methods:** From January to July 2001, 298 Type 2 diabetic patients were consecutively recruited from 61 primary care establishments in Moscow city and the Moscow region. Exclusion criteria: age < 40, newly diagnosed diabetes, inability to read and understand Russian, unwillingness to participate. There were 60 males and 238 females, aged 60.4 ± 10.1 (range, 41–83), with diabetes duration of 9.2 ± 6.7 (range, 1 to 32) ys. All patients were assessed for acute and late complications of diabetes, HbA1 c (normal < 6.2%), BMI, antidiabetic medication. Validation consisted of 3 steps: a) linguistic validation, including forward and back translation b) collection of data: patients filled in the questionnaire after they had been explained the purpose of the study c) psychometric validation, which included assessment of reliability, internal consistency and validity of the Ru-ADDQoL.
**Results:** Each of the 18 domain-specific ADDQoL items was relevant and important for substantial numbers of patients. There was a predominantly negative effect of diabetes on all aspects of life. Positive influence of diabetes was noted for 12/18 items in a small number of patients, namely, Q12 "future" (33/298 patients, 11.1%), Q14 "dependence from others" (11/298, 3.6%) and other items (<1%). Forced one-factor analysis (factor loading > 0.4 for all items) and Cronbach's $\alpha$ coefficient of internal consistency (0.93) supported combination of 18 items into a scale. This was confirmed by two types of analysis: with missing values excluded and, more conservatively, with missing values replaced by zero. Insulin-treated patients perceived greater negative impact of diabetes on their QoL, than non-insulin-treated, the mean weighted QoL scores being respectively -2.9 ± 1.6 and -2.1 ± 1.9 ($p < 0.001$, ranked ANOVA). Patients who did not report any hypoglycaemia within the last month (n=257) had significantly higher mean weighted QoL score (-2.2 ± 1.62), than those with hypoglycaemia (n=41) (-3.03 ± 1.9, p=0.008, ranked ANOVA). There was a significant difference in the mean weighted QoL scores in patients who reported to have no diabetic complications or to be unaware of them (-1.86 ± 1.63, median -1.5, n=60) and those who were aware of their diabetic complications (-2.51 ± 1.72, median -2, n=205, p = 0.0046, ranked ANOVA). The number of complications *known* to a patient correlated with the mean weighted QoL score (r = -0.2, p=0.0016). Mean weighted QoL scores correlated well with the rating of Qol if without diabetes (r = 0.56, p<0.0001) and correlated weakly but significantly with the present general QoL rating (r = 0.18, n=298, p=0.002).
**Conclusion:** the linguistic and psychometric validation of the Ru-ADDQoL showed its validity and reliability as a diabetes-specific instrument for the Russian-speaking adults with diabetes.

## 924

**Effects of living arrangements and cognitive function on metabolic control in older patients with Type 2 diabetes mellitus**
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**Background and aims:** We investigated whether living arrangements and cognitive impairment affect metabolic control in elderly patients with type 2 diabetes mellitus (DM).

**Materials and methods:** 167 (98F/69M) patients with type 2 DM aged 70 yrs and over (age ;76.2 ± 0.9 yrs, BMI;19.9 ± 0.5 kg/m$^2$) were studied. They were subdivided into group I, 60 patients living alone, and group II, 107 patients living with family members. Cognitive function was assessed with Hasegawa dementia rating scale (HDS). Glycemic control was assessed by HbA1 c. Diabetic retinopathy, nephropathy and neuropathy were evaluated by fundoscopic examination by ophthalmologist, urinary albumin excretion rate, and clinical neurological examinations. Cardiovascular disease (CVD) was assessed by history of coronary artery disease and stroke, as well as ischemic changes in ECG and pulse wave velocity (PWV).
**Results:** Age was similar between the 2 groups. HbA1 c (%) was higher in the group I (7.66 ± 0.16) than in the group II (6.28 ± 0.48)(P<0.01). The prevalence of diabetic retinopathy and neuropathy was higher in the group I. The prevalence of nephropathy and CVD did not differ between the 2 groups. Patients with dementia in the group I (7.93 ± 0.41 % vs 6.94 ± 0.66 % , P<0.01) or II (6.81 ± 0.11 % vs 6.32 ± 0.21 % , P<0.05) had higher HbA1 c than the nondemented counterparts. The patients with dementia had higher blood pressure (BP) than those without (148.6 ± 0.6/88.5 ± 0.7 vs 136.2 ± 0.5/80.7 ± 0.5, P<0.05) in the group I. However, dementia did not affect BP in the group II. The prevalence of retinopathy and neuropathy was higher and PWV (cm/s) was faster in the patients with dementia in the group I (2096.5 ± 0.5 vs 1896 ± 0.4, P<0.05). No such differences were noted in the group II.
**Conclusion:** Living arrangements and cognitive function affect glycemic control and BP in older patients with type 2 DM. Older patients with dementia living alone are most vulnerable to diabetic complications and CVD.

## 925

**Cognitive function and peripheral polyneuropathy in patients with Type 2 diabetes mellitus**
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The pathopysiology of cognitive dysfunction and peripheral polyneurathy in patients with diabetes mellitus is still unclear..
**Aim:** To assess if patients with type 2 diabetes mellitus and peripheral polyneuropathy (PPN) show cognitive dysfunction more often than patients without PPN.
**Methods:** A neuropsychological examination (premorbide, current: Trail-Making-Test A und B, Number-Symbol-Test, Mosaictest, MWT) was performed in 102 patients with type 2 diabetes mellitus (Age 68,6 ± 8,7y., diabetes duration 10,3 [0,03–35,4]y., Body-Mass-Index (BMI) 28,7 ± 5,8 kg/m$^2$, HbA1 c 10,3 ± 1,7%, Diamat® NR:4,4–5,9%) to assess cognitive function. The examination of polyneuropathy was performed according to Young et al. The current cognitive function (CCF) was 87,7 ± 12,3 IQ-points and premorbide cognitive function (PCF) 96,3 ± 9,2 IQ-points (n= 102).
**Results:** 48 of 102 (47,1%) examined patients had a peripheral polyneuropathy, 54 (52,9%) had no PPN. There were no statistically significant differences in both groups concerning age, HbA1 c, blood pressure and BMI. But patients with PPN showed longer diabetes duration (Patients with vs. without PPN: 15,3 [0,7–35,4] vs. 7,4 [0,03–30,6]y., p=0,004). Current and premorbide cognitive function were comparable in both groups (Patients with vs. without PPN: CCF: 87,9 ± 12,1 vs. 87,5 ± 12,5 IQ-points, p=0,87; PCF: 97,4 ± 9,6 vs. 94,4 ± 8,9 IQ-points, p=0,29). The multivariate analysis showed an association of cognitive dysfunction and elevated HbA1 c-levels (R-square=0,08, β=–0,28, p=0,005). Age, diabetes duration, blood glucose and systolic blood pressure were not associated with cognitive function.
**Conclusion:** The peripheral polyneuropathy is not associated with impaired cognitive function in patients with diabetes mellitus.

## 926

**Promoting physical and psychological wellbeing among Samoan people with diabetes in New Zealand: Does a good doctor make a difference?**
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**Background and aims:** Previous research has shown psychological factors at an individual level (for example health beliefs) can play an important role in determining diabetes behaviours. However little attention has been given to the impact interpersonal variables, such as relationship with health professionals, may have on diabetes self care. This study examined the role of doctor-patient relationship in promoting physical and psychological wellbeing among Samoan people with type 2 diabetes in New Zealand.
**Materials and methods:** Data was collected through structured interviews. These were used to complete an English language questionnaire (N=70, response rate 60%). Inclusion criteria for this study were (a) diagnosed with type 2 diabetes, (b) aged over 18 and (c) self-identify as Samoan (indigenous to the South Pacific).
**Results:** Research findings showed relationships with health professionals are an important determinant of diabetes self-management behaviour and psychological wellbeing. Using linear regressions doctor-patient relationship was found to explain a significant proportion of variance in diabetes self-care. Specifically, doctor patient relationship was found to predict 17% of variance in medication use (R2 adjusted =0.17, p<0.01), 30% of differences in foot care (R2 adjusted =0.30, p<0.01), 32% of exercise habits (R2 adjusted =0.32, p<0.01) and 49% of variation in eating patterns (R2 adjusted =0.49, p<0.01). Doctor-patient relationship was also found to explain 26% of variation in diabetes specific distress among the sample (R2 adjusted =0.26, p<0.01).
**Conclusions:** Findings of the present study suggest doctor-patient relationship can be important in determining self-management and psychological wellbeing among people with diabetes. This highlights the need for theoretical models that incorporate interpersonal factors as predictors of diabetes self-care. Results suggest good interpersonal skills are an essential part of clinical care in diabetes. These can be used to promote positive patient self-care behaviours and, through this, positive clinical outcomes. Research findings illustrate the significance of interpersonal facets of health care delivery. They may serve to remind us of an „important and oft-forgotten phenomenon, the therapeutic potential of human interactions."
*The author wishes to acknowledge the support provided to this project by a Claude McCarthy Fellowship.*

## 927

**Quality of life in subjects with and without Type 2 diabetes mellitus**
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**Background and aims:** Type 2 diabetes mellitus is a chronic and progressive disease with a negative impact on quality of life. Objectives of the present study were to describe Health- Related Quality of life (HRQOL) in type 2 diabetes mellitus and to compare the health state between diabetic and non-diabetic subjects.
**Materials and methods:** Type 2 diabetes mellitus patients were selected from a representative sample of the Italian general population aged from 40 to 79 years enrolled in a population based naturalistic prospective survey. We matched each of them by age and sex with a non-diabetic subjects. The EuroQoL (EQ-5D), a self-administered generic questionnaire, completed during the enrolment visit, was used to evaluate HRQOL.
**Results:** We analyzed two groups of 157 subjects each (diabetic and non-diabetic group). The mean age was 63.0 years, 94 (59.9) were male. Diabetic patients reported more problems than non-diabetic subjects in the physical sphere, specifically for mobility and usual activities (P = 0.027 and P = 0.006 respectively), while in self care, pain/discomfort and anxiety/depression dimensions, there was no statistically significant difference between the two groups. Mean values of the visual analogue scale assessing global health status indicated by patients with and without type 2 diabetes mellitus were 70 (SD, ± 16.92) and 72 (SD, ± 16.75), respectively (P = 0.395).
**Conclusion:** This study, comparing diabetic and non-diabetic patients of the same age and sex, suggest that the presence of type 2 diabetes mellitus is associated with higher problems in the physical sphere, specifically in domains such as mobility and usual activities, but not on the overall perception of health status.

## 928

**Evaluating quality-of-life in persons treated with insulin versus oral agent therapy who present with characteristics of latent autoimmune diabetes in adults (LADA)**
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**Background and aims:** Epidemiological studies have shown that the incidence of type 1 diabetes is bimodal, peaking at puberty and again at age 40. Often this peak in mid-aged individuals is not appreciated because of the nearly 10-fold greater incidence of type 2 as compared to type 1 diabetes in this age group. Moreover, non-obese individuals aged 30–55 years presenting with hyperglycemia and relative insulin deficiency and controlled initially on oral agents are clinically distinct from older, obese, insulin resist-

ant patients with type 2 diabetes. These clinical characteristics are also prognostic of Latent Autoimmune Diabetes in Adults (LADA), which typically exhibits with antibodies characteristic of type 1 diabetes including ICA and GADA and rapidly leads to insulin dependency. The purpose of our cross-sectional study was to evaluate the impact of insulin versus oral therapy on quality of life in subjects with characteristics predictive of LADA.
**Materials and methods:** We identified 163 individuals with typical characteristics of LADA (BMI < 30 kg/m$^2$, age = 30–55 yrs, c-peptide = 0.1–0.6 nmol/l) from a baseline database of 8 pooled clinical trials of type 1 and type 2 diabetes with a total of 3,727 participants. We compared patients using insulin only (INS) to those using oral agents only (OA) by examining Overall QOL (scaled 100 – 600) and its 10 psychological and physical subscales. Between-group differences were evaluated using linear regression models after adjusting for covariates of HbA1 c, age, and duration of diabetes.
**Results:** Patients had a mean ± SD age = 46 ± 6 yrs, HbA1 c = 8.9 ± 1.4%, BMI=25.9 ± 2.5 kg/m$^2$ and diabetes duration = 8.8 ± 6.7 yrs. 85% had a diagnosis of type 2 diabetes and 15% type 1 diabetes; 43% were on INS (2 or more injections per day) vs. 57% on OA or diet only. The INS group was younger (45 vs. 47 yrs), had lower HbA1 c (8.0 vs. 9.6%), both P < 0.001, and longer duration of disease (11 vs. 7 yrs, P = 0.046) compared to the OA group. For both groups combined higher HbA1 c was associated with worsened mental health (r = 0.16, p = 0.049), particularly psychological distress (r = 0.19, p = 0.02) and loss of emotional and behavioral control (r = 0.2, p = 0.17), and greater symptom distress (r = 0.17, p = 0.04). After adjustment for covariates, Overall QOL (mean ± SE) was still significantly worse for INS vs. OA (457 ± 9 vs. 483 ± 8, P=0.04). The difference was primarily driven by the QOL scale of General Health Perceptions (vitality, health status, and sleep) where insulin users scored significantly worse than non-users (443 ± 10 vs. 489 ± 9, P=0.003).
**Conclusion:** In individuals with typical characteristics of LADA, Overall QOL was lower among those receiving insulin, even after controlling for HbA1 c, age, and duration of diabetes. The findings suggest an underlying physical as well as psychological differential between the two treatment modalities. Since the majority of LADA patients are initially treated for type 2 diabetes, screening patients for age, BMI, and c-peptide suggestive of LADA, and performing confirmatory tests for ICA or GAD antibodies, might enable clinicians to prepare high risk individuals for the physical, behavioral and psychological adjustments required with eventual insulin therapy.

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## 929

**Locus of control in patients with Type 2 diabetes after long-term management by group care**
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**Background and aims:** The locus of control (LoC) describes the relationship between the patients' behaviour and various daily life situations. We have developed and validated a model to manage type 2 diabetes by systemic group education (Group Care) which resulted in sustained body weight reduction, increased HDL-cholesterol and stabilization of HbA1 c along with improved health conducts, knowledge of diabetes and quality of life. To investigate the LoC in patients with type 2 diabetes followed for 5–7 years by Group Care compared with others followed by traditional one-to-one care.
**Materials and methods:** Two questionnaires were administered to 56 patients followed by Group Care and 51 controls randomized by age, sex, duration, glycaemia, insulinaemia, weight and other clinical and socio-economic variables. Patients on Group Care had lower HbA1 c (7.40 ± 1.21) than the controls (7.99 ± 1.48), p=0.027. Both questionnaires include 18 items assessing 3 areas: internal control of disease, the role of chance in changing the disease, and trust in health operators. The Peyrot and Rubin (PR) questionnaire is specific for diabetes. The Wallston and Wallston (WW) questionnaire explores a wide range of situations and is considered more generic for chronic diseases.
**Results:** Both questionnaires showed lower scores for Chance in patients followed by Group Care, while Trust did not differ from those followed by traditional care. Only the PR tool showed increased Internal Control in the patients followed by Group Care. Multivariate analysis showed that the HOMA index of insulin resistance was inversely related to Internal Control independently of BMI and HbA1 c.

| | PR Internal Control | PR Chance | PR Trust | WW Internal Control | WW Chance | WW Trust |
|---|---|---|---|---|---|---|
| Group Care | 31.8 ± 4.1 | 15.0 ± 5.6 | 28.1 ± 5.1 | 29.1 ± 5.0 | 16.3 ± 5.5 | 28.9 ± 6.1 |
| Control | 28.8 ± 6.5 | 28.2 ± 2.0 | 28.0 ± 4.6 | 29.2 ± 4.6 | 27.1 ± 5.8 | 28.9 ± 5.8 |
| Significance | p<0.001 | p<0.001 | NS | NS | p<0.001 | NS |

**Conclusion:** Group Care appears to reduce fatalistic attitudes and to increase internal control without modifying trust in the operators in patients with type 2 diabetes. These changes appear to influence insulin resistance, above and beyond the effects of body weight and metabolic control.

## 930

**Why people smoke? The case of persons with diabetes**
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**Background and aims:** Given the serious health consequences of diabetes and smoking and the well-established cost-effectiveness of smoking cessation in general, it is quite surprising that there has not been more attention given to smoking cessation in diabetes research and practice. Among the general population, several studies have shown that physicians counseling during a simple routine consultation increases the likelihood that the patient will stop smoking. On contrary, a number of studies that have evaluated the effectiveness of intervention with diabetic smokers did not have very optimistic results. The purpose of this study was to evaluate the smoking habits in persons with diabetes and to identify the psychological factors related to smoking status.
**Materials and methods:** During standard ambulatory visits, 72 smokers with type 2 DM (mean age = 53.1+/–8.89 yrs, duration of DM = 4.46 yrs) were asked to complete a 17-items questionnaire, assessing the smoking

habits, the motives and beliefs regarding smoking, the craving and control smoking behaviors, the knowledge level about the smoking consequences and the desire to quit. Demographic, clinical and laboratory data were also colected.
**Results:** The smoking pattern in people with diabetes is characterized by an increased nicotine addiction, revealed by early lighting up and more cigarettes been smoked during the morning (62.5% of subjects) and a decreased control over smoking behavior: smoking more than wanted ($p<.03$), neglecting responsabilities due to smoking, smoking when sick ($p<.02$), or forbidden ($p<.003$) etc. The influence of psychosocial factors on smoking behavior appeared to be significant, with increased smoking when socializing ($p<.02$) and with perceived benefits of smoking on stress relief and induced relaxation ($p<.03$). The fear of weight gain is also contributing to continuing smoking, especially in women ($p<.004$). The knowledge level on smoking consequences over diabetes progression and management is surprisingly low and the contribution of health care team on smoking cessation is perceived as insufficient.
**Conclusion:** The nicotine addiction in smokers with diabetes is similar with that in general population. The main psychological contributors on continuing smoking are the perceived smoking benefits on reducing negative emotional states, facilitating socialization, considering smoking as more social desirable than alcohol consumption, fear of weight gain. The present findings underline the necessity of identifying the attitudinal and behavioral components of smoking prior and during any smoking cessation intervention program in people with diabetes. The results sustain the importance of including the smoking status as „vital sign" in the routine consultation in order to enhance the physician's time and resources spent with smokers with diabetes.

## 931

**Development and empiric analysis of a standardized questionnaire to assess treatment satisfaction after participation in structured treatment and teaching programmes**
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**Background and aims:** To assess treatment satisfaction with structured treatment and teaching programmes (TTP) for patients with diabetes mellitus a standardized questionnaire was developed and checked for psychometrical characteristics.
**Materials and methods:** 94 patients with type 2 diabetes mellitus (Age 68.6 ± 8.4y.; diabetes duration 10.3 [0.03–35.4]y., HbA1 c 10.3 ± 1.7%, gender: 53.2%f/46.8%m), who participated in an inpatient structured TTP for insulin therapy, answered the treatment satisfaction questionnaire immediatly after TTP. This questionnaire implies twelve 5-stepped likertscaled items and comprises questions about subjective overtaxation and treatment competence. The sum score was used for evaluation (Range: 12–60, high values represent high satisfaction). Afterwards psychometric characteristics and validation was checked.
**Results:** The questionnaire shows good reliabilty/internal consistance (Spearman-Brown: r=0.86; Cronbachs $\alpha=0.87$) and satisfactory good selectivity of items. The distribution of scores is normal $p=0.09$, quartiles 45.0 – 50.5 – 55.0).
**Conclusion:** This questionnaire is a psychometric adequate measuring instrument to assess treatment satisfaction with structured treatment and teaching programmes. Further evaluations in different clinical cohorts (different age, type 1 diabetes) would be useful to check especially external validity.

## 932

**Development and validation of a clinical screening instrument for identification of Type 2 diabetes patients with reduced cognitive abilities**
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**Background and aims:** Type 2 diabetic patients are at greater risk for significant cognitive decline because of diabetes-related and diabetes-unrelated factors (e.g. age). The availability of therapeutic regimens varying in their complexity and their demands poses the problem of the identification of T2DM patients with reduced cognitive abilities. Conventional screening instruments stemming from neurology/neuropsychology are often not particularly helpful because most instruments aim at the detection of severe impairments/dementia and they are felt to be discriminating by many patients. Aim of this study was the development and validation of a cognitive screening test for older T2DM patients suitable for broader clinical application.
**Materials and methods:** The screening test was constructed stressing to the following principles: (1) test economy; (2) acceptance/diabetes self-care related tests. It includes five subtests: (1) picture sorting; (2) arithmetics; (3) blood glucose log interpretation; (4) memory; (5) vigilance. Reliability and validity of the instrument were studied in a clinical sample of older T2DM patients (n=84, age 67.9 ± 5.6 yrs.; duration of disease 15.1 ± 9.8 yrs.; HbA1C 8.5 ± 1.4%). To study validity, the dementia screening DemTect and the WAIS-R intelligence test battery were administered as reference instruments.
**Results:** Item analysis yielded satisfactory to good results. Internal consistencies of the subtests were $\alpha=0.66$ to $\alpha=0.81$ (Cronbach), reliabilities (Spearman-Brown) were $r=.65$ to $r=.92$. The whole test was internal consistent and reliable ($\alpha=.87$; $r=.94$), scores being approx. normally distributed (Kolmogorov-Smirnov test $p=.307$). Significant correlations with the WAIS-R and DemTect were observed ($r=.68$, $r=.55$).
**Conclusion:** The newly developed tests is a screening instrument with sound psychometric properties suitable for broader clinical application to identify diabetes patients with reduced cognitive abilities that could hamper self-care behaviour, though further studies on its sensitivity/specifity and clinical utility (i.e. potential benefit with regard to treatment decisions) are needed.

## 933

**Patient-physician relationship: a therapeutic tool in overcoming psychological insulin resistance.**
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**Background and aims:** The psychological insulin resistance is a concept that defines patient's reluctance to initiate or intensify insulin treatment. Previous studies have analyzed the impact of patient-physician relationship upon various aspects of treatment adherence. This study aims to explore patient-physician relationship as a contributing factor to psychological insulin resistance.
**Materials and methods:** This study is cross-sectional, comprising 45 participants with type 2 DM, that have either refused or accepted insulin therapy, as proposed and explained by the diabetes physician. The 2 groups (group 1: 23 persons who refused insulin, group 2: 22 persons who accepted the therapy) were analyzed using qualitative and quantitative psychological measures, assessing: emotional status, locus of control, previous proximal (inside patient's family or social group) experience with insulin therapy, comorbidities, HbA1 c, health beliefs regarding diabetes, the necessity to start insulin therapy, and attitudes towards health-care team. The results were analyzed using SPSS 11.0 software.
**Results:** The 2 groups did not differ significantly in mean duration of diabetes (group 1: mean =10.2 years, group 2: mean=11.1 years), presence of complications, HbA1 c (where available) or emotional status. The results of the variance analysis (ANOVA) show that the factors that best differentiate the two groups are: previous proximal experience with insulin therapy ($p=0.03$), self-blame ($p=0.001$), as well as the quality of the therapeutic relationship ($p=0.02$). Those patients that perceived the physician as inexperienced were more likely ($p<0.001$) to be amongst those who refused the treatment. Also, the patient's belief that he has been involved in the therapeutic decision regarding insulin treatment initiation increased the likelihood of patient to accept insulin therapy.
**Conclusion:** This study underlines the importance of the therapeutic relationship in adherence to insulin treatment. These findings suggest that the psychological insulin resistance can be influenced by the interpersonal relationships themselves. Since this study is cross-sectional, post-decisional cognitive effects might bias the attitudes measured. Further interventional prospective studies are needed in order to clarify the importance of this relationship in increasing patient's adherence to a treatment whose beneficial effects upon health-status and well-being have been already established.

## 934

**Drivers of treatment preference among individuals with Type 2 diabetes**

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**Background and aims:** Patient preferences are crucial in treatment decision-making when several equally efficacious alternative treatments are available. The objective of this study was to investigate the principal drivers of treatment preference among individuals with type 2 diabetes.
**Materials and methods:** We conducted 18 focus groups with 158 adults with type 2 diabetes (36% on orals only, 33% on insulin oral combination, 26% on insulin alone) supplemented with treatment preference driver checklists. The first 5 focus groups yielded 10 drivers of treatment preference. The second 13 focus groups ranked the importance of the 10 drivers among 100 points. Summary statistics were calculated for each driver and correlation coefficients were calculated among the driver rankings.
**Results:** Average age of participants was 54. Two-thirds were female and 37% were African American. Considerable variability existed in scores attached to each driver. Two drivers received scores from both extremes of the continuum (i.e., 13% of respondents ranked medication effectiveness as 0 and 2% ranked it as 100; 45% of respondents ranked financial costs as 0 and 1% ranked it as 100). The mean and median scores for each driver are presented in Table 1 below. Rankings of medication effectiveness were significantly ($p < 0.05$) and inversely correlated with rankings of emotional side effects ( $r = -0.26$), treatment tolerability ($r = -0.24$), and physician recommendation ($r = -0.23$). Following the initial ranking, respondents were asked to assume medication effectiveness was perfect and then to re-rank the remaining nine drivers. In this round of scoring the rank order of drivers changed only slightly. Physical side effects had the highest mean ranking (20.2) followed by financial costs (12.4), emotional side effects (11.9), lifestyle/quality of life impact (11.2), treatment flexibility (10.5), correct dosing (9.3), treatment tolerability (7.9), physician recommendation (7.9), treatment convenience (7.6).
**Conclusion:** Great variability exists in the drivers of treatment preference among individuals with type 2 diabetes. Group averages mask tremendous inter-individual variability in the importance of drivers and their relative rank. These findings underscore the need for continued methodological work on the concept of treatment preference.

Table 1. Rankings of Drivers for Diabetes Treatment Preference

| Treatment Preference Drivers | Mean Ranking (N=119) | Median Ranking (N=119) |
|---|---|---|
| 1. Medication effectiveness | 29.0 | 23.0 |
| 2. Physical side effects | 13.5 | 9.0 |
| 3. Financial costs | 8.9 | 3.0 |
| 4. Emotional side effects | 8.1 | 3.0 |
| 5. Treatment flexibility | 7.1 | 3.0 |
| 6. Lifestyle/ Quality of life impacts | 7.2 | 0.0 |
| 7. Correct dosing | 6.3 | 0.0 |
| 8. Physician recommendation | 6.0 | 0.0 |
| 9. Treatment tolerability | 4.2 | 0.0 |
| 10. Treatment convenience | 3.6 | 0.0 |

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## 935

**Course of depression in Type 2 diabetes**

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**Background and aims:** Whereas there are consistent findings about an elevated prevalence of depressive disorders in diabetes, less is known about the course of depression in diabetic patients. In a prospective study we examined the course of depression and it's possible predictors in type 2 diabetic patients.
**Materials and methods:** 191 type 2 diabetic patients took part in a prospective trial to evaluate the efficacy of selfmanagement programs in a 15-month follow up. At the beginning and the end of this follow up 177 patients (HbA1 c 7.8 ± 1.6%, age 55.5 ± 6.5 yrs.; 50.6% female, BMI 32.2 ± 3.8 kg/m$^2$) completed a depression questionnaire (Zerssen Depression Scale; drop out 7.9%). Patients who scored higher than 10 (1 SD > mean) in the depression scale were considered as depressed.
**Results:** At baseline 54 patients (30.5%) reached an elevated depression score. From these 28 patients (15.8%) improved to an asymptomatic level, whereas 26 patients (14.7%) remained depressed at follow up. Incidence of depression was 5,1% (9 patients). In a exploratory logistic regression analysis female gender (OR 4.9 CI 1.7–14.0), number of late complications (OR 1.9 CI 1.1–3.6) and amount of weight loss (OR 1.4 CI 1.0–2.1) differentiated significantly between patients, who stayed depressed from patients who remained non depressed. In a second logistic regression only the number of complications (OR 4.8 CI 1.7–13.3) differentiated significantly patients who remained depressed from patients who recovered from depression. A smaller decrease in HbA1 c (OR 0.6 CI 3.4–1.1, P=.08) showed a tendency towards significance.
**Conclusion:** After participation in diabetes education a great proportion of initially depressed patients recovered. Late complications seem to hamper recovery, thus coping with late complications should be specifically addressed. Interestingly a greater amount of weight loss was associated with persistence of depression, indicating that changing eating habits may be perceived as a burden. A smaller improvement in HbA1 c showed a weak association to persistence of depression, which points to the need for further research on the link between glycemic control and depression.
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## Genes and hormones in pregnancy

### 936

**Insulinotropic effect of gastric inhibitory polypeptide (GIP) in women with a history of gestational diabetes**

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**Background and aims:** The insulinotropic effect of GIP is reduced in patients with type 2 diabetes and at least 50 % of their first degree relatives at hyperglycemic conditions. It is unknown, whether this is due to a specific defect in GIP action or a general reduction in B-cell function. Therefore, the insulinotropic effect of GIP was studied in women with previous gestational diabetes (pGDM).
**Materials and methods:** 20 pGDM women and 20 controls were studied with the intravenous bolus administration of 20 pmol GIP/kg body weight, and with an oral glucose tolerance test. A hyperglycemic clamp experiment (140 mg/dl over 120 min) with the intravenous infusion of GIP (2 pmol $.kg^{-1}. min^{-1}$ from t = 30 to 90 min) was performed in 14 women in each group. Capillary and venous blood samples were drawn for the determination of glucose (glucose oxidase), insulin, C-peptide, GIP and GLP-1 (specific immunoassays). Indices of insulin sensitivity and B-cell function were calculated. Statistics were carried out using repeated-measures ANOVA and Duncan`s *post hoc* tests.
**Results:** Following oral glucose ingestion, plasma glucose, insulin and C-peptide concentrations rose to higher levels in the pGDM women than in controls ($p < 0.05$). The secretion of GLP-1 and GIP was similar in both groups ($p = 0.87$ and $p = 0.57$, respectively). The bolus administration of GIP led to an increase in insulin secretion ($p < 0.001$), but no differences were found between both groups ($p = 0.99$ and $p = 0.38$, for insulin and C-peptide, respectively). There were no differences in the insulin secretory response to GIP administration during the hyperglycaemic clamp experiment between the pGDM women and the controls ($p = 0.88$ and $p = 0.82$, for insulin and C-peptide, respectively). The pGDM women were characterised by a higher degree of insulin resistance than controls ($p = 0.007$ for the Matsuda index), but they exhibited no defects in glucose-stimulated insulin secretion ($p = 0.40$ for the insulinogenic index calculated during the hyperclycemic clamp experiment). There was a hyperbolic relationship between the insulin secretory response to the GIP bolus administration and the Matsuda-index, indicating a compensatory increase in GIP-induced insulin secretion in response to higher levels of insulin resistance.
**Conclusion:** The fact that women at high risk for type 2 diabetes with a predominant defect in insulin action show a preserved insulin secretory response to GIP does not support the hypothesis of a primary insulin secretory defect regarding GIP-action in these subjects and the development of type 2 diabetes in general. A reduced insulinotropic effect of GIP in patients with type 2 diabetes may rather reflect a general reduction in B-cell function in these patients. A hyperbolic relation between insulin resistance and the insulin secretory response to either glucose or GIP points to a B-cell problem determining the secretory response to both stimuli.

### 937

**Circulating resistin and TNFα levels are increased, while adiponectin is decreased, in normal and gestational diabetes pregnancy**

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**Background and aims:** Adipose tissue derived hormones and cytokines have been suggested to be important regulators of insulin resistance. In humans, both TNFα and resistin are also expressed in peripheral monocytes and in the placenta. We examined whether circulating adiponectin, resistin and TNFα levels are altered in normal and in gestational diabetes (GDM) pregnancy and whether this might relate to the hyperinsulinemia of these conditions.
**Materials and methods:** We studied 19 women with normal pregnancy (Gest), 15 with gestational diabetes (GDM), and 28 non-pregnant controls, all premenopausal. All pregnant women were studied during the 3rd trimester. We measured plasma resistin (BioVendor Inc), plasma adiponectin and TNFα (R&D Systems) by Elisa. We also measured glucose and insulin (RIA) levels at 0, 60 and 120 min after an OGTT.
**Results:** Plasma resistin and TNFα levels were significanly increased, while plasma adiponectin levels were decreased in both pregnant groups (normal and GDM) compared to controls (Table). No clear differences were observed between the groups of normal and GDM pregnancy, which both had significantly higher fasting and post-OGTT insulin levels compared to controls, suggestive of insulin resistance.

| | BMI Kgr/m² | GluAUC† mg/dL | InsAUC† mIU/L | Resistin ng/ml | Adiponectin ng/ml | TNFα pg/ml |
|---|---|---|---|---|---|---|
| Control | 27.5 ± 1.3 | 212 ± 8 | 68 ± 9 | 2.96 ± 0.18 | 13.00 ± 1.39 | 2.17 ± 0.13 |
| Gest | 26.7 ± 1.4 | 250 ± 8* | 168 ± 28* | 6.46 ± 0.57* | 10.91 ± 1.52 | 3.10 ± 0.28* |
| GDM | 25.7 ± 1.0 | 334 ± 19*§ | 180 ± 23* | 5.56 ± 0.39* | 9.24 ± 1.01* | 3.30 ± 0.28* |

*, $p<0.05$ vs control, §, $p<0.05$ vs. Gest., , Area under the curve

**Conclusion:** Circulating resistin and TNFα levels, most probably deriving from the placenta, are significantly increased, while adiponectin levels are decreased in normal and gestational diabetes pregnancy. This might contribute to the insulin resistance that characterises these conditions and might increase their atherogenic risk.

### 938

**Maternal serum ghrelin levels in healthy pregnant women and gestational diabetes in correlation with cytokines and insulin resistance**

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**Background:** Ghrelin is a 28-amino acid peptide, mainly produced and secreted by the mucosal enteroendocrine X/A cells of the stomach (and placenta during pregnancy). The protein has numerous endocrinological activity, among them it increases the production of different pituitary hormones and insulin, food intake and weight gain. In humans ghrelin prodution was suppressed by insulin.
**Aim:** In a cross sectional study the fasting ghrelin concentration was measured in healthy pregnant women (15 in each trimester, n=45), patients with gestational diabetes (GDM, n=30, each of them treated with insulin), and in age-matched healthy nonpregnant control women (n=30) in correlation with some indirect parameters of insulin resistance (fasting C-peptide level, C-peptide/blood glucose ratio) and cytokine levels infuencing insulin sensitivity (TNFα, its soluble receptors R1 and R2 and leptin).
**Materials and methods:** Fasting serum biologically active ghrelin (Linco) and C-peptide (Biodata) concentrations were detected by RIA; TNFα (Sigma), sTNF-receptor (R)-1, -2 (Bender MedSystem) and leptin (DRG) levels were measured by ELISA. Statistical analysis was performed by using Prism3 program.
**Results:** Serum ghrelin levels were significantly higher in healthy pregnant women in the 2nd (376 ± 38 pg/ml, X±SD) and lower in the 3rd trimester (251 ± 35), and in patients with GDM (226 ± 21), as compared to pregnant women in the 1st trimester (313 ± 40) and healthy nonpregnant control women (309 ± 20, $p<0.01$, Mann-Whitney). Significant ($p<0.01$, Spearman) negative linear correlations were calculated arnong ghrelin levels, BMI values and fasting C-peptide concentrations in each groups; and between ghrelin concentrations and fasting C-peptide/blood glucose ratios, TNFα, sTNFR-2 and leptin levels in the GDM and healthy pregnant groups.
**Conclusion:** The increased ghrelin concentration at the beginning of pregnancy, mainly in thc 2nd trimester may increase food intake and contribute to the weight gain of the pregnant women. In the ara trimester of pregnancy the increasing insulin resitance, insulin, TNFα and leptin levels may downregulate the production of the protein. A negative regulatory mechanism limiting the further increase of the body mass in that period of pregnancy is suggested.
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## 939

**Hyperleptinemia in neonates of mothers with gestational diabetes**

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**Background and aims:** Leptin is a hormone produced by adipocytes and placenta, that has been directly related with neonatal growth. The purpose of this study was to compare leptin levels in umbilical cord blood of large-for-gestational-age newborns of mothers with gestational diabetes with a control neonates of nondiabetic mothers.
**Materials and methods:** Cord blood leptin concentrations were measured in newborns of 30 women (24 control neonates and 06 gestational diabetes mellitus). Birth weights were measured with a calibrated scale and leptin levels were measured by radioimmunoassay (Linco Research, St Charles, MI, USA).
**Results:** There was no significant difference in birth weight between the control neonates and neonates of mothers with gestational diabetes (3950 ± 39.96 g versus 4090 ± 120.46 g; p=0.496). Cord leptin concentrations were significantly greater in neonates of mothers with gestational diabetes mellitus than in control neonates (30.07 ± 6.17 ng/mL versus 12.43 ± 2.02 ng/mL; p=0.004), with no gender difference (p>0.05). In all subjects, cord leptin was significantly correlated with birth weight (p=0.000). Therefore, when an adjustment was made for birth weight and sex, there was a significant diference in cord leptin concentration between control neonates and neonates of mothers with gestational diabetes mellitus (p=0.003).
**Conclusion:** These findings confirm the relationship between leptin levels and birth weight and also demonstrate that neonates of mothers with gestational diabetes had higher leptin concentrations than the nondiabetic group, although there was no birth weight difference between these groups. This hyperleptinemia observed in neonates of mothers with gestational diabetes may be mediated through altered maternal glucose metabolism and hyperinsulinism, up-regulating the production of adipose tissue characteristic of these infants.
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## 940

**Gender differences in serum leptin concentrations from umbilical cord blood of newborn infants of ND mothers and offspring of Type 2 diabetic and GDM mothers at delivery**

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**Background and aims:** Gender difference in the leptin concentration in the newborn babies is still a controversial issue. The present study has been undertaken to address the issue by measuring leptin concentration in the cord blood of new born babies from nondiabetic (ND group), Gestational diabetic (GDM group), Type 2 Diabetic (DM group) mothers.
**Materials and methods:** Serum leptin concentrations were measured in ND newborns (male = 25 and female = 25, DM newborns (male = 25 and female = 25), GDM newborns (male = 25 and female = 25). Maternal anthropometric measurements were recorded at admission for delivery. Both serum leptin and serum C-peptide level was measured by chemiluminescence- based ELISA.
**Results:** The leptin concentration (ng/ml, mean ± SD) in cord blood was ND male 12.99 ± 12.01, female 21.84 ± 10.40, DM male 27.36 ± 15.03, female 37.35 ± 16.78, GDM male 35.42 ± 12.96, female 42.84 ± 15.13. Serum leptin concentrations of female babies of ND, Type-2 DM and GDM groups were higher compared to the male babies of the same groups. Birth weight of female babies of ND, Type 2 DM and GDM groups were also higher compared to the male babies of the respective groups. ND male 2.85 ± 0.359, female 3.09 ± 0.31, DM male 3.10 ± 0.49, female3.52 ± 0.73, GDM male 3.16 ± 0.65, female 3.61 ± 0.46. Serum leptin concentrations of female babies of ND, Type-2 DM and GDM groups were also higher compared to the male babies of the same groups. Serum leptin concentrations in cord blood were positively correlated with birth weight in each group In ND Male (r = 0.674, p<0.0001), Female (r=0.537, p=0.006), In DM Male (r=0.606,p<0.001), Female (r = 0.511, p=0.009), GDM Male (r= 0.707, p<0.0001), Female (r = 0.611, p<0.001). The serum C-peptide concentrations did not correlate with the leptin concentration in any of the groups.
**Conclusion:** Sex difference exists regarding leptinemic status at birth with higher values in female. The higher serum leptin levels, however, seems to be related to be a function of higher birth weight of the female babies.

## 941

**Leptin concentrations in cord blood: relationship to fetal growth and maternal anthropometry of GDM mothers at delivery**

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**Background and aims:** Cord blood leptin may reflect the leptinemic status of a newborn at birth more accurately than the leptin values of blood collected from other sites. The present study was undertaken to determine the relationship of cord serum leptin concentrations at birth with neonatal and maternal anthropometric parameters.
**Materials and methods:** Blood was taken from the umbilical cord of the babies at delivery. Maternal anthropometric measurements were recorded at admission for delivery. Neonatal anthropometric measurements were recorded within 48 hours after delivery. Linear regression analysis was used to explore the relationship between serum leptin concentrations and anthropometric parameters of the baby and the mother.
**Results:** The leptin concentration (ng/ml, mean±SD) in cord blood was 39.13 ± 14.44. Cord leptin levels correlated with birth weight (r=0.673, p<0.0001), Ponderal index (r=0.732, p<0.0001) and mid-arm circumference of the mother (r=– 0.415, p=0.003), but it did not correlate with gestational age (r= 0.135, p=0.349)at delivery, cord serum C-peptide concentration (r= –0.049, p=0.735) or placental weight (r= 0.203, p=0.157) .
**Conclusion:** There are associations between cord leptin concentrations at delivery and birth weight and Ponderal index of the babies and mid-arm circumference of the mother. High leptin levels of the baby could represent an important feedback modulator of substrate supply and subsequently for adipose tissue status during late gestation.

## 942

**Relationship of leptin to carbohydrate metabolism, c-peptide, insulin, fat mass, BMI and weight in 32 pregnant women**

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**Background and aim:** The relationship between the appearance of Gestational Diabetes Mellitus (GDM) and leptin were discussed in several studies. The role of leptin in the interaction with other hormones during pregnancy is still not explored. A comparison between women with GDM and healthy pregnants showed a significant difference of leptin in the maternal plasma. We investigated healthy pregnants in a longitudinal survey during pregnancy and evaluated the changes in leptin concentration.
**Material and methods:** Our study included 32 pregnant women (29 nondiabetics, 1 GDM, 2 pregnants with impared glucose tolerance) before the 16$^{th}$ gestation week. The patients received a CGMS® (MinimedMedtronic®) in the 16$^{th}$, 22$^{nd}$, 30$^{th}$ and in the 36$^{th}$ gestation week and 6 weeks after delivery up to 72 hour. The sensor was calibrated seven times a day by a portable blood glucose device (Accu-Check®, Roche®, Mannheim, Germany). AT each visit were carried out an oral glucose tolerance test (OGGT), measured c-peptide and insulin and determined the body composition with the body impedance analysis (BIA) (Data Input GmbH, Frankfurt, Germany).The diagnosis of GDM and Impaired glucose tolerance (IGT) was made by an oral glucose tolerance test rated by the recommendation of the German Diabetes Association.
**Results:** The mean age was 29,6 ± 4,5 years (Min 19; Max 39 years). In the 16$^{th}$ gestation week the BMI was on an average 24,0 ± 2,6 kg/m$^2$ (min 18,0; max 29,2 kg/m$^2$). From the 16$^{th}$ to 30$^{th}$ gestation week Leptin rose statistically significantly and decreased 6 weeks postpartum. Until the 36$^{th}$ gestation week body weight, BMI, fat mass, insulin and c-peptide also rose statistically and decreased 6 weeks postpartum. One of these 32 pregnant women developed a GDM during our investigation, two of them an impaired glucose tolerance. Those patients did not present elevated leptin levels compared to the control group.
The correlation analysis showed a strong relationship between leptin and the BMI, the weight and the fat mass in each part of investigation.
Following markers for insulin resistance were observed: fasting c-peptide, fasting insulin and fasting c-peptide/glucose. C-peptide and c-peptide/glucose showed a significant correlation with the leptin levels in the 22$^{nd}$ and 30$^{th}$ gestation week. Insulin only showed a significant correlation with lep-

tin in the 22nd gestation week. Blood glucose correlated with plasma leptin level only in the 30th gestation week.
**Conclusions:** Normoglycaemic pregnant women showed no clear associations between blood glucose and leptin levels. The leptin levels depends more on the BMI, fat mass and bodyweight. Other studies have shown a difference in pregnant women with and without carbohydrate intolerance. This effect is not reproducible in healthy pregnant women. Maternal leptin correlated at different gestational age with the fat mass, body weight and BMI, but not with the blood sugar, c-peptide and insulin.

## 943

**Haptoglobin phenotype and gestational diabetes**
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**Background and aims:** Haptoglobin (Hp), a hemoglobin binding plasma protein, exists in two major allelic variants. Hp 1 has higher hemoglobin binding and antioxidant capacity compared to Hp 2. Gestational Diabetes (GDM) is usually confined to the time of gestation, but women with prior GDM carry an increased risk to develop DM 2 and associated vasculopathy later in life.
**Materials and methods:** 251 Caucasian women were tested for GDM (24<sup>th</sup>- 28<sup>th</sup> gestational week, 75 g OGTT) and their Hp phenotype was determined (Electrophoresis on semi-automated Pharmacia PhastSystem, detection by rabbit anti human Hp-/goat anti rabbit-IgG). Significance of distribution as well as Odds Ratio associated with Hp phenotype were calculated for cases (impaired oral glucose tolerance, IGT) and controls (normal glucose tolerance, NGT).
**Results:** 111 women had an IGT (43% had 1, 44% had 2, 13% had 3 elevated plasma concentrations of glucose), 140 women had NGT. Of the 111 women with IGT, 8 (7%) women carried dimeric Hp exclusively (Hp1-1 phenotype), 44 (40%) had dimeric and oligomeric Hp (Hp 1-2 phenotype) and 59 (53%) had oligomeric Hp only (Hp2-2 phenotype). Respective values for women with NGT were 32 (23%), 57 (41%) and 51 (36%). Homozygous Hp phenotype 2, was significantly more frequent in cases than controls (53% vs. 36%, p
<0,01). The Odds ratio for gestational diabetes in women homozygous for Hp 2 was 1,98 (CI 95%: 1,19–3,3).
**Conclusion:** The relatively low antioxidative potential of Hp 2 and its predominant occurrence in GDM might be a genetic link between oxidative damage and insulin resistance. Homozygous Hp 2 phenotype is therefore an apparent risk factor for the development of GDM.

## 944

**Candidate gene search for gestational diabetes mellitus in Arabian and Scandinavian women: association with genetic variations in genes involved in β-cell function**
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**Background and aims:** Impaired pancreatic β-cell function and insulin resistance are the hallmark of gestational diabetes mellitus (GDM). In the present study we investigated whether common polymorphisms in genes affecting insulin secretion and action are operative in GDM and whether the genotype frequencies differ between Arabian and Scandinavian women.
**Materials and methods:** In total 500 unrelated GDM women (400 Scandinavian and 100 Arabian) and 550 unrelated pregnant non-diabetic controls (428 Scandinavian and 122 Arabian) were genotyped for the E23K polymorphism in the pancreatic β-cell ATP-sensitive $K^+$ ($K_{ATP}$) channel subunit Kir6.2, Gly972Arg polymorphism in the insulin receptor substrate-1 (IRS-1) and SNP+276G/T variant in the adiponectin (APM1) genes using the TaqMan 5' allelic discrimination assay or RFLP method.
**Results:** Among Arabian women, the Kir6.2 K/K genotype was more frequent in GDM compared to controls (15 vs. 5.7%, p=0.02) whereas no significant difference was seen between Scandinavian GDM and controls (15 vs. 13.8%, p=0.23). The IRS-1 Arg972-allele frequency was similar in GDM and controls both in Arabians (9.5 vs. 7.0%, p=0.3) and Scandinavians (6.1 vs. 5.6%, p=0.7). However, in Scandinavian GDM women, Arg972-allele carriers had lower HOMA β-cell index (0.22 ± 0.02 vs. 0.39 ± 0.04, p=0.02) compared to Gly/Gly-genotype carriers. The T-allele frequency of the APM1 SNP+276G/T variant did not differ between GDM and controls neither in Arabians (33 vs. 29.1%, p=0.4) nor in Scandinavians (29.9 vs. 26.7%, p=0.2). However, in Arabian GDM women, T-allele carriers had higher fasting serum insulin (14.3 ± 1.6 vs. 8.9 ± 0.8, p=0.02) compared to G/G-genotype carriers.
**Conclusion:** We show that the Kir6.2 E23K polymorphism is associated with GDM among Arabian group whereas the IRS-1 Arg972-allele is associated with impaired β-cell function in Scandinavian GDM women. These data suggest that genetic variations affecting pancreatic β-cell function may contribute to GDM in these populations.

## 945

**Comparison of lipid metabolism parameters in pregnant women with diabetes of Type 1 with and without microangiopathy**
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**Background and aims:** Hyperglycemia and dyslipidemia are the causes of angiopathy development in diabetes of type 1. In pregnancy we can observed the microangiopathy intensification. The aim of the study was the estimation of lipid parameters in pregnant women with diabetes of type 1.
**Materials and methods:** Forty three women were examined. In 34 of them the microangiopathy complications were not observed before pregnancy - the B and C classes according to White (group A). However, those complications were observed in 9 patients - the D and R classes (group B). Twenty seven non-pregnant women with diabetes of type 1 constituted the control group (21 patients without complications - CGA and 6 with microangiopathy - CGB). Lipid parameters: total cholesterol (TC), HDL, LDL and triglyceride (TG) as well as apolipoprotein (Apo A) and apolipoprotein (Apo B) were estimated at the end of every trimester of pregnancy in both groups while in CG the estimation was made during admitting the patients to the hospital. Glycemic control in pregnancy was estimated by means of mean blood glucose (MBG), fructosamine (F) and $HbA_{1C}$ in both groups and by means of $HbA_{1C}$ in CG. Glycemia was determined by using glucose oxydase. The TC, LDL, HDL, TG concentrations were determined with the enzymatic method by means of the Bio Merieux firm sets (France), the Apo $A_1$ and Apo B-100 - with the immunoturbidymetric method by using reagents of the Boeringer Mannheim sets (Germany). $HbA_{1C}$ was determined with immunoturbidymetric method using the Roche firm sets (France).
**Results:** Glycemic control was very good and there were no significant differences between the analyzed groups ($HbA_{1C}$ in A group was 5,9+/–0,9, in B - 6,2+/–0,6, in CG - 6,3+/–0,6%). The TC, TG, LDL concentrations were statistically higher in the first trimester (T1) in B group than in A group (TC - 5,5+/–1,4 vs 4,3+/–0,7, p<0,001; TG - 1,2+/–0,7 vs 0,9+/–0,4, p<0,05, HDL - 66,67+/–22,28 vs 64,57+/–18,32 and LDL - 2,9+/–1,0 vs 2,3+/–0,6, p<0,05). The lipid metabolism parameters were higher in CGB than in CGA, too (TC - 5,1+/–0,6 vs 4,4+/–0,8, p<0,05; TG - 1,1+/–0,3 vs 0,8+/–0,4, p<0,05 and LDL - 0,3+/–0,7 vs 2,5+/–0,8, p<0,05). There were no differences in the remaining lipid metabolism parameters. The TC and LDL increase was bigger in A than in B during pregnancy and those values were similar at the end of pregnancy. The Apo B value in pregnancy in the B group was higher than the LDL value (Apo B - 73% and LDL - 13%) in comparison to the A group (Apo B - 77% and LDL - 47%). In the A group the HDL parameter statistically increased in pregnancy (T1 - 64,57 + / - 18,32 vs T2 - 78,67+/–14,31, p<0,01 and T1 vs T3 - 77,35+/–18,96, p<0,01). However, in the B group after significant increase of HDL in T2, decrease of HDL value was observed in T3. Apo A increased in every trimester in both groups. The TG increase in pregnancy was even in both groups.
**Conclusion:** 1. Despite very good glycemic control the lipid metabolism changes were observed. Women with diabetes of type 1 with complications had higher TC and LDL values especially in the first trimester and higher TG values during the whole period of pregnancy. 2. In women with diabetes complications the increase of Apo A in every trimester was higher than the increase of LDL, which can be a result of changing the structure of LDL particle into a more aterogenic one.

# PS 89

# Training and structure in diabetes care

## 946

**Type 1 diabetes as a „Tracer" condition in developing countries**

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**Background and aims:** To assess the prevalence and patterns of care of type 1 diabetes in 2 sub-Saharan African countries and to relate prevalence, and estimates of life expectancy, to geographical factors and availability of insulin and monitoring equipment.
**Materials and methods:** Using a Rapid Assessment Protocol developed by the IIF to collect information from government organisations and from central, regional and peripheral health units, as well as patients and their carers. Cross checking between different data sources was used to establish validity. Between 100 and 200 interviews/discussions were undertaken in each country.
**Results:** The overall prevalence of type 1 diabetes was around 5 per 100,000 in Mozambique and 11 per 100,000 in Zambia. Estimates of prevalence in Mozambique differed by around 7-fold between the capital city and the rural area, but these differences were only twofold in Zambia. Insulin was available in sufficient amounts at the national level in both countries, but supplies to peripheral units were more variable, particularly in Mozambique, with patients having to purchase insulin, at substantial expense, from private pharmacies. Most health units in Mozambique lacked any means of measuring either blood or urine glucose, for diagnostic or monitoring purposes. Knowledge of type 1 diabetes care was poor among most health workers in Mozambique.
**Conclusion:** Type 1 diabetes is associated with poor outcomes in some parts of sub-Saharan Africa, particularly away from urban hospitals. There is, nevertheless, evidence that health worker training, combined with advocacy by patients' associations, can impact on services and outcomes, and that the Rapid Assessment process can act as a catalyst to improving diabetes care. The research also highlighted that increasing the supply of insulin alone will not lead to improved conditions for patients. Improvements in health care systems, pharmaceutical supply, and health worker training are important to tackle Type 1 diabetes. These improvements will be likely to benefit care not only for Type 1 diabetes, but also for other non-communicable and communicable diseases.
*Supported by: World Diabetes Foundation, World Health Organisation, Diabetes Foundation*

## 947

**Comparison of health care services' utilization and metabolic control between diabetic Ethiopian and non-Ethiopian patients**

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**Background and aims:** The cost of medical care for Israeli Ethiopian immigrants has been found to be significantly lower than for Israelis in the general population. This may indicate under-utilization of health care services by the Ethiopian population, despite an estimated relatively high prevalence of both diagnosed and undiagnosed chronic illnesses likely to occur during the acculturation period of new immigrants from non-Western cultures. This study focused on the healthcare provided to Ethiopian origin (EO) immigrants for diabetes mellitus, a disease that was nearly non-existent in this population in Ethiopia, but became relatively prevalent after immigration. The first objective was to examine the quality of medical treatment and metabolic parameters in EO patients with diabetes. The second one was to identify obstacles for effective health services' utilization by EO patients regarding diabetes & cardiovascular risk factors.
**Materials and methods:** Seventy six EO diabetic patients were randomly selected from five primary care clinics of Clalit Health Services (HMO) in the city of Natanya, which absorbed many EO immigrants. As a control group, each EO patient was matched with two same-aged non-Ethiopian origin (NEO) patients. Patients were interviewed in their mother's tongue regarding quality of communication with the medical team and services' utilization. Medical data, recorded the year prior to the initial data gathering, were collected from the patients' files.
**Results:** Significant differences were found in the quality of follow-up between EO patients with diabetes and the controls. Interview data: The following parameters' rates were significantly lower among EO than in NEO patients - understanding physician's explanations (48% *vs* 92%, $p<0.001$); consultation with the nurse regarding diabetes (26.5% *vs* 46.6%, $p<0.001$) and understanding pharmacist's instructions (18.1% *vs* 79.2%, $p<0.001$). The need for assistance when examined by the physician was higher among EO diabetics (31.1% *vs* 15.3%, $p<0.001$) . Less EO patients reported having blood pressure measurements (66.2% *vs* 91.7%, $p<0.001$), feet examination (27.4% *vs* 61.0%, $p<0.001$) and fundus examination (54.9% *vs* 72.2%, $p<0.001$). Patient file data: The rate of measurements of the following parameters was lower in EO diabetics – blood pressure; performance of eye examination; laboratory tests including HbA1C, serum creatinine and lipoprotein profile. HbA1C was 9.6% ± 2 in the EO patients versus 8.1% ± 1 in controls ($p=0.02$).
**Conclusion:** Means to address cultural and economic barriers of EO patients to healthcare services need to be developed in order to ensure equity in health care services' utilization.
*Supported by: The Israel National Institute for Health Policy and Health Services Research (NIHP)*

## 948

**Disease-specific medical records improve processes of care in the management of Type 2 diabetes mellitus**

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**Background and aims:** The quality of care for patients with diabetes mellitus has been shown to be variable and sub-optimal wherever it has been studied around the world. Over the last ten years the Tunisian Ministry of Health have gradually instituted a national program of hypertension and diabetes management within primary care health centres (PHCCs) that includes the use of disease-specific medical records. This study seeks to test the hypothesis that the introduction of these medical records has improved the documentation of care.
**Materials and methods:** Retrospective medical record review of a two stage, randomised sample of patients with type 2 diabetes from the northern regions of Tunisia. Data regarding patient characteristics, process of care and outcome of care criteria were collected from all patient visits to the health centres in 2000, 2001 and 2002. Documentation of care in the new disease-specific medical records was compared with the previous general medical records.
**Results:** 964 patient records were randomly selected from twenty-six randomly selected PHCCs distributed throughout the north of Tunisia. Data from 7930 visits to the health centres were collected; 3980 were recorded in the new medical records and 3950 in the standard medical records. The proportion of visits recorded in the new medical records increased from 37% in 2000 to 60% in 2002. Recording of all the process of care measurements was significantly higher in the new disease-specific medical records compared to the standard general medical records. For example, blood pressure was measured in 84% of visits in the new medical records compared to 65% of visits in the standard records ($p<0.001$, $\chi^2=265$). The corresponding figures for weight measurement were 25% and 3% ($p<0.001$, $\chi^2=876$). These results were consistent for each of the three calendar years when analysed independently and also for the sub-group of patients who changed from using the standard records to the new records during the time frame of the study. No significant differences were found in the characteristics of patients for whom care was recorded in the standard records, new records or both types of records during the 3-year period. Intermediate outcome variables showed no differences between the three groups.
**Conclusions:** We have confirmed the hypothesis that the introduction of disease-specific medical records significantly improves the documentation of care of patients with type 2 diabetes mellitus. We would recommend that all countries, particularly those without the resources to use computerised systems, consider introducing disease-specific medical records for the management of their patients with chronic diseases.

## 949

**Patient Individualised Application of Research Evidence in clinical diabetes care: The πr Project**

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**Background and aims:** The last few years have seen the publication of multiple randomized clinical trials and important reviews. These can serve to provide an evidence-base to reduce morbidity and mortality in patients with diabetes. With the explosion in clinical knowledge it is difficult to remain appraised of relevant research papers and their clinical application. A simple clinical tool, **PIARE** (Patient Individualised Application of Research Evidence in Clinical Diabetes Care) was developed to allow clinicians to use evidence from clinical trials/reviews effectively in clinical diabetes care.
**Materials and methods:** The clinical phenotype of the case-patient was compared to the study population characteristics. The PIARE Tool gave a score which showed the studies matched most closely to the patient phenotype based on age, sex, blood pressure, cholesterol, microalbuminuria, retinopathy status, etc. Twelve clinical landmark papers in diabetes care: UKPDS 33, UKPDS 34, UKPDS 38, 4S, HPS, Micro-HOPE, RENAAL, EUCLID, LIFE, IRMA, STENO-2 and ADA Aspirin Recommendations were used in this project. Fifteen clinicians (11 doctors, 3 specialist nurses and 1 pharmacist) were given 10 cases each to comment upon with respect to clinical management. The case was shown initially with the details of the case alone and the clinicians made recommendations. This was repeated with synopses of relevant studies predicted from our PIARE Tool. A simple scoring system allowed the investigators to calculate the level of valid/correct clinical decisions before and after using the PIARE Tool.
**Results:** On the initial assessment of the case histories, only 46 percent (0–87 percent) were able to apply the clinical trials appropriately. There was a significant difference in correct treatment scores, pre-PIARE 69 percent and post-PIARE 98 percent ($p<0.001$).
**Conclusion:** PIARE 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. This tool will be made freely available for use in clinical practice.

PIARE Tool results

| | Clinician aware of trials | Trials used in practice | Pre-PIARE score | Post-PIARE score |
|---|---|---|---|---|
| Average | 64% | 46% | 69% | 98% |
| Range(%) | 25–100 | 0–100 | 0–87 | 97–100 |

## 950

**Cardiovascular risk prognosis for informed decision making – validity of prediction tools**

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**Background and aims:** Patient involvement in health care decisions is increasingly requested. We investigated whether currently available assessment tools for prediction of cardiovascular risk can be used for individual risk prediction as a basis of informed decision making.
**Materials and methods:** We searched for risk assessment tools and respective validation studies in Medline (until 2/2004) and the Cochrane Library (Issue 1/2004). The following criteria were used for evaluation of prognostic studies: 1) discrimination between risk groups; 2) predictive values; 3) prognostic agreement 4) transferability across populations.
**Results:** A total of 12 assessment tools were identified. The Framingham-function, Sheffield-Tables, Framingham Categorial-, New Zealand-, Joint British-, and European Charts (1994 and 1998) are based on the Framingham-Study; PROCAM-Risk-Score, UKPDS Risk Engine, and SCORE-Tables use different source data. Framingham-based instruments overestimate cardiovascular risk of central- or southern-European populations by at least 30%, with substantial regional variation even within a country (between 30 and 200%, British Regional Heart Study). Therefore, prior to application the assessment tools would need recalibration using regional data of cardiovascular mortality and adjustment for social class differences. Sensitivity, specificity, and C-statistic for external validation (AUC approx. 0.6) are clearly inferior to internal validation (AUC approx. 0.8). Agreement between instruments beyond chance is moderate (kappa approx. 0.5). No studies on external validation could be identified for the new European SCORE-Tables and UKPDS Risk Engine.
**Conclusion:** Validation of currently available assessment tools for cardiovascular risk prediction is inadequate. Uncritical use may lead to substantial under- or overestimation of individual cardiovascular risk and inappropriate treatment decision.

## 951

**Long-term evaluation of the treatment and teaching programme for Type 1 diabetic patients in Moscow (13-years results)**

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**Background and aims:** Patient education and self-monitoring is regarded as the basis for a improvement of metabolic control, reduction of diabetes-related complications. The aim of the study was to evaluate the long-term results of structured programme for treatment and teaching of Type 1 diabetic patients (DTTP).
**Materials and methods:** The programme consisted of training in small groups of 6 to 10 patients for 5 hospital days. 64 Type 1 diabetic patients (28 male, 36 female; age 27–60 yrs, mean 41.7 ± 8.5 yrs (mean±SD); diabetes duration 6–43 yrs, mean 23.0 ± 7.4 yrs) were re-examined after DTTP during follow-up period of 13 yrs. Total group of diabetic patients was divided two subgroups (SG I, SG II). Patients of SG I (n=24) visited study centre every 4–6 months during follow-up period and had regular telephone contacts. These visits included elements of individual education: review and adjustment regimens of insulin therapy, improvement knowledge and skills, discussion of urgent problems and etc. Patients of SG II (n=40) were treated in routine system of public health without intensive follow-up.
**Results:** Mean HbA1 c before DTTP was 9.6 ± 0.8% (normal up to 6.4%). There was a significant improvement of metabolic control 1 yr after DTTP (see table), SG I was not differ from SG II. HbA1 c level increased in 7 yrs and went back to baseline in 13 yrs, but it was significantly lower in SG I. The frequency of diabetic ketoacidosis (DCA) fell down from 0.19 cases/patient/yr before DTTP to 0 ($p<0.001$) 1, 7 and 13 yrs after. The frequency of severe hypoglycaemia (SH) was 0.08 cases/patient/yr before DTTP, 0.09, 0.12 and 0.13 (NS) in 1, 7 and 13 yrs of the follow-up, respectively. Diabetes related hospitalization (DRH) was 9.6 days/patient/year before DTTP, it decreased to 0.7, 0.9 and 2.8 ($p<0.001$) 1, 7 and 13 yrs after, respectively. There was not significant difference in frequency of DCA and SH, duration of DRH between SG I and SG II. 83% patients carried out glycaemia self-monitoring, mean rate of measurements in total group was 15.7 ± 14.4 per week. All patients of SG I performed self-monitoring compared 75% in SG II. Patients of SG I did measurements more frequently: 26.1 ± 17.7 vs 10.3 ± 9.5 per week in SG II ($p<0.001$).
**Conclusion:** This study demonstrated high effectiveness of DTTP in lowering frequency of acute complications and duration of DRH during long-term follow-up. However metabolic control was acceptable only in subgroup with regularly re-examinations. Therefore, intensive continuous follow-up should be integrated in standard diabetes care after DTTP.

| HbA1 c, % | Total group | SG I | SG II | p SG I vs SG II |
|---|---|---|---|---|
| Baseline | 9.5 ± 1.0 | 9.4 ± 1.2 | 9.6 ± 0.8 | 0.628 |
| 1 yr after DTTP | 7.5 ± 1.0 | 7.1 ± 1.2 | 7.6 ± 0.9 | 0.086 |
| p 1 yr vs baseline | <0.001 | <0.001 | <0.001 | |
| 7 yrs after DTTP | 8.7 ± 2.5 | 7.7 ± 1.1 | 9.3 ± 2.9 | <0.002 |
| p 7 yrs vs baseline | 0.027 | <0.002 | 0.521 | |
| 13 yrs after DTTP | 9.1 ± 1.9 | 8.0 ± 1.1 | 9.7 ± 2.0 | <0.001 |
| p 13 yrs vs baseline | 0.161 | <0.002 | 0.776 | |

## 952

**Can the single-episode of therapeutic patient education improve glucose metabolism indicators?**

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**The aim** of this study was to determine of effectiveness of individual and group therapeutic patient education as a type 2 diabetes therapy element.
**Material and methods:** 80 type 2 diabetic patients aged under 75 yr., with history of diabetes up to 1 yr., treated with insulin twice daily, who has never undergone structural diabetes education were enrolled into the

study. The patients were randomly divided into two groups depending on the type of education: A (n=40) – individual, B (n=40) – group. Age of patients, duration of diabetes, BMI, HbA1 c, fasting and postprandial glycemia were comparable in both groups. All patients underwent three-day structural diabetes education programme. At the beginning and at the end of education test of diabetes knowledge was checked. After 3 months patients completed the same test. Fasting and postprandial glycemia (just after education and 3 months later), BMI and HbA1 c (3 months after education) were evaluated.

**Results:**

| | Group A | | | Group B | | |
|---|---|---|---|---|---|---|
| | At the beginning of education programme | At the end of education programme | 3 months later | At the beginning of education programme | At the end of education programme | 3 months later |
| Knowledge [points] | 25,4±2,3 | 27,9 ± 1,4*** | 23,8± 1,4*** | 26,7±2,7 | 26,8±2,3† | 26,1±2,0 |
| BMI [kg/m²] | 29,2±3,1 | | 27,8±2,9 * | 28,2±5,0 | | 27,5±4,8 |
| Fasting glycemia [mg/dl] | 136±48 | | 122 ± 27 | 131±44 | | 117± 26 |
| Postprandial glycemia [mg/dl] | 195±57 | | 166±35** | 192 ± 64 | | 171±53 |
| HbA1 c [%] | 6,7±0,7 | | 6,5 ± 0,5 | 6,8 ± 0,9 | | 6,6±0,7 |

* p<0,05; ** p<0,01; ***p< 0,000001 towards former parameter

**Conclusion:** 1. Individual therapeutic diabetes patient education gives better results than group therapeutic education. 2. Individual therapeutic diabetes patient education is a relevant element of diabetic therapy, which improves metabolic control in type 2 diabetic patients. 3. Metabolic effects of individual therapeutic education persist longer than knowledge effects due to education programme 4. Short individual diabetes education programmes have only short-time influence on patient's knowledge about diabetes which doesn't last for a long time and decreases with the lapse of time.

## 953

**Knowledge, attitude and practice of hypercholesterolemic Type 2 diabetic subjects on dyslipidemia**

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**Background and aims:** Dyslipidemia is a common problem in type 2 diabetic subjects. Studies related to knowledge, attitude and practice (KAP) of diabetic subjects on dyslipidemia are relatively rare in developing countries. The present study is the first one in Bangladesh on this issue.The aim of the study is to assess the knowledge, attitude and practice of newly diagnosed hypercholesterolemic type 2 diabetic subjects on dyslipidemia and to analyze the influence of some demographic and socioeconomic factors on the level of KAP.

**Materials and methods:** Ninety seven newly diagnosed type 2 diabetic subjects (male:female 61:36, age 46 ± 9 years, mean±SD) with hypercholesterolemia (fasting plasma total cholesterol >200 mg/dl) were selected from the Out-Patient Department of BIRDEM. Data were collected by a predesigned, pretested, interviewer-administered questionnaire. The subjects were graded as low, medium and high as follows: knowledge-score <50%, 50%–60% and 60%; attitude-score <60%, 60%–80% and 80%; and practice-score <50%, 50%–70% and 70% respectively.

**Results:** The levels of knowledge were low in 31%, medium in 33% and high in 36% of the study subjects. The corresponding attitude levels were low in 2%, medium in 59% and high in 39%, and the levels of practice were low in 72%, medium in 21% and high in 7% of the subjects. The subjects of urban, semi-urban and rural area did not significantly differ on knowledge and practice. The subjects of urban, semi-urban and rural area significantly differ on attitude (77.5 ± 9.7% vs 83.9 ± 5.2% vs 74.4 ± 8.7, P<0.05). Compared with illiterate-primary group (52.2 ± 10.3%) knowledge score was high in secondary (57.5 ± 10.3%, P<0.05), graduate and post-graduate (58.7 ± 11.6%, P<0.05) groups. Practice score of illiterate-primary group (35.7 ± 28.7%) was lower than secondary group (45.2 ± 19.9%), graduate and post-graduate groups (50.6 ± 32.4%, P<0.05), but they did not differ on attitude. Attitude score of high-income group {median (range)} of the subjects was {79 (59–94.2)} better than low {63.8 (58.1–84.7)} and medium income group {77 (65.7–98.1)}, P<0.05). Compared with high-income group practice score {44.3 (16.6–150)} was low in medium income group {25 (0–66.6)} and low income group {16.6 (5.5–83.3)}, P<0.05), but they did not differ on knowledge.

**Conclusion:** Knowledge, attitude and practice, the three important components of awareness, do not follow a one to one relation in the newly diagnosed hypercholesterolemic type 2 diabetic subjects. The variation is largely explained by demographic, educational and socioeconomic factors, which determine the outcome in a complex fashion. Thus, a coordinated development policy is required to promote knowledge and attitude on healthy diet and to translate those into practice.

# PS 90

# Cost-effectiveness in diabetes care

## 954

**Costs of Type 2 diabetes mellitus: a comparison between diabetic and non-diabetic subjects**

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**Background and aims:** Type 2 diabetes mellitus is a common chronic disease and a costly health care problem. Its prevalence is expected to increase in the future, especially in developing countries. The aims of this study were: 1) to assess the social costs of type 2 diabetes mellitus and 2) to evaluate the costs of diabetic patients in comparison with non-diabetic subjects.
**Materials and methods:** We conducted a Cost of Illness (COI) analysis from a societal perspective with a 3 month time horizon. Data were collected from a population based naturalistic prospective survey, designed to investigate cardiovascular risk factors in a sample of the Italian general population aged from 40 to 79 years. We selected all type 2 diabetic patients and we matched each of them by age and sex with a non-diabetic subject. Patients were interviewed by general practitioners about clinical/demographic characteristics, medical resource utilization, absence from work and reduced working activity during the 3 months before the enrolment visit. Direct medical costs were quantified including hospitalizations, drug therapies, specialist visits, diagnostics and laboratory exams, while indirect costs were estimated based on productivity losses with the Human Capital approach.
**Results:** We studied 666 patients, 333 with type 2 diabetes mellitus matched with 333 without the disease. In both groups, the mean age was 63.1 years, 192 (57.7%) were male. The mean total cost per patient-month was Euro 228.7 compared to Euro 169.9 for patients with and without type 2 diabetes mellitus, respectively (P<0.0001). On average, direct medical cost per patient-month was estimated at Euro 199.2 in diabetic patients and Euro 129.1 in non-diabetic subjects (P<0.0001). Hospitalizations accounted for the greatest proportion of healthcare costs in both groups, followed by drug therapies (hospitalizations: 65.1% and 59.6%; drug therapies: 24.5% and 29.7% in patients with and without type 2 diabetes mellitus, respectively). There was no statistically significant difference in indirect costs between diabetic and non-diabetic subjects.
**Conclusion:** The results of our analysis show that type 2 diabetes mellitus patients aged from 40 to 79 years are more costly than non-diabetic subjects.

## 955

**Long-term cost-effectiveness of the Diabetes Prevention Program in an Italian setting**

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**Background and aims:** In the Diabetes Prevention Program (DPP), interventions with metformin (plus standard lifestyle advice) or intensive lifestyle changes (ILC) reduced the risk of developing type 2 diabetes by 31% and 58%, respectively, versus control in subjects with impaired glucose tolerance (IGT). We have adapted a peer-reviewed, published diabetes prevention model to assess the cost-effectiveness of DPP interventions in an Italian setting.
**Materials and methods:** The published Markov model simulated 3 states: "IGT", "type 2 diabetes", and "death". Probabilities were derived from the DPP and published Italian data. Life expectancy (LE) was calculated for each treatment arm. Italian-specific direct costs of implementing DPP interventions and of diabetes were retrieved from published sources. Total costs/patient (TC) and costs/life-year gained were calculated. LE was discounted at both 0 and 5% annually, while costs were discounted at 5% annually in accordance with current Italian guidelines. Extensive sensitivity analyses were performed.
**Results:** Both interventions improved LE but increased TC versus control. Mean improvements in non-discounted LE were 0.34 and 0.90 years for metformin and ILC, respectively. TC were increased by € 1,136 and €2,761/patient with metformin or ILC respectively, with incremental costs/life-year gained (TC and LE discounted 5% annually) of €11,044 and €11,360 for metformin or ILC versus control, respectively. Results were most sensitive to the probabilities of developing type 2 diabetes, the relative risk of mortality for type 2 diabetes compared with IGT, and the costs of implementing ILC.
**Conclusion:** Metformin and ILC were both highly cost-effective. The initial cost of pharmacological or lifestyle-based intervention in prediabetic individuals should not deter healthcare systems from implementing diabetes prevention programs, as both interventions represent excellent value for money when compared to other currently reimbursed medical interventions.
*Supported by: Merck-Santé Lyon, France*

## 956

**Complications extend total annual medical cost of Type 2 diabetes**

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**Objectives:** Diabetes imposes a large economic burden on NHS and society. In order to investigate the medical cost attributable to type 2 diabetes we conducted a retrospective longitudinal cost of care study in a diabetologic center in Italy.
**Methods:** We *a priori* estimated to validly enroll 300 type 2 diabetic patients with a follow-up of at least one year. To this aim, we randomly selected 315 patients from a base of approximately two thousands diabetic patients attending the diabetologic center of Portogruaro (Venice) during the period January 2001–August 2002.
Cost included hospitalizations, visits, diagnostics and pharmacological therapies, and were quantified in the perspective of the National Health Service.
We extracted clinical and demographic information from the electronic database and performed extensive charts review, including comorbidities: retinopathy, cardiopathy (coronary heart disease), vasculopathy (other than CHD) and nephropathy.
We analysed the association between diabetes-related comorbidities and average annual medical costs using univariate and multiple linear regression analyses.
**Results:** Two-hundred-ninety-nine patients were at least considered for this analysis and followed-up for an average 476 days, totalling 520 person years of observation. Their mean age was 68,6 years (SD8,8); 201 (67,2%) were male.
The average annual cost of care was Euro 1909,67; 52% of costs was attributable to drugs, 28% to hospitalisations, 11% to diagnostics and 9% to visits.
Patients free of diabetic-related comorbidities were 101 (33,8 %), 117 (39,1%) had one and 81 (27,1%) had two or more complications. The more frequent complication was vasculopathy affecting 89 (29,8%) patients, followed by cardiopathy 79 (26,4%), retinopathy 66 (22,1%) and nephropathy 65 (21,7%). The annual medical costs increased with the number of complications from Euro 1.039,59 to 1.808,17 and to 3.141,21 in patients with none, one and two and more complications respectively, the association being statistically significant both in univariate (Kruskall-Wallis Test =73,035, $p$<0.0001) and in multiple linear regression analyses (P<0.0001, $R^2$=0,21, F Test =82,5, P<0,0001).
**Conclusions:** Long term complications have an impact on total annual medical cost. Our study demonstrate that the increase of comorbidities number is directly associated with an increase of type-2 diabetes cost. Strategies aimed at preventing the onset of diabetic complications are likely to reduce medical costs in the long run, while improving patients' health.

## 957

**Cost-effectiveness analysis of insulin glargine compared with NPH insulin based on a 10-year simulation of long-term complications with the diabetes mellitus model in patients with Type 2 diabetes in Switzerland**

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**Background and aims:** All new pharmaceutical products must meet acceptable thresholds of cost-effectiveness set by each country in order to be

approved for use in general practice. The objective of this study was to evaluate the cost effectiveness of insulin glargine (LANTUS®) compared with NPH insulin in patients with Type 2 diabetes in Switzerland.
**Materials and methods:** Long-term diabetes outcomes were simulated over a period of 10 years with the Diabetes Mellitus Model (DMM). The incidence of long-term complications, (micro- and macrovascular events), were simulated for 10,000 patients over 10 years for nine different scenarios. Scenarios were based on $HbA_{1c}$ reductions observed in clinical trials or obtained from meta-regression analyses. For insuline glargine $HbA_{1c}$ reductions of 0.93% (worst case), 1.24% (base case), 1.65% (best case) were simulated for three different $HbA_{1c}$ baseline values (10%, 9% and 8%). For NPH insulin the $HbA_{1c}$ reduction was assumed to be 0.8%. Unit costs of micro- and macrovascular events were based on published cost estimations and guideline-projected resource use for Switzerland. Total direct medical costs were assessed by a combination of cumulated incidences of each event up to 10 years with the corresponding unit cost per event in addition to the cost of therapy. All cost analyses for insulin glargine versus NPH insulin were performed on a per-patient level. In scenarios where no savings could be gained by insulin glargine, incremental cost effectiveness (ICE) values were calculated as incremental cost per microvascular, macrovascular or total event prevented.
**Results:** Cost minimisation analysis yielded savings in six scenarios ($HbA_{1c}$ 10%, 9% and 8% for best case and base case scenarios) (table). Cost savings per patient ranged from CHF5909 for $HbA_{1c}$ 10 % best case to CHF160 $HbA_{1c}$ 8% medium case. In the base case analysis, savings in the management of complications exceeded the difference in acquisition cost in years 6, 7 and 9 of treatment. Worst case scenarios for all three baseline $HbA_{1c}$ values yielded no cost savings, but ICEs of CHF27,630, CHF29,719 and CHF35,374 per patient and event prevented were achieved for 8 %, 9 % and 10% baseline $HbA_{1c}$, respectively.
**Conclusion:** $HbA_{1c}$ reductions of 1.65% and 1.24% with insulin glargine compared with 0.8% with NPH insulin are sufficient to yield total cost savings per patient starting with a baseline $HbA_{1c}$ of 10%, 9% and 8%. Therefore insulin glargine is a cost-effective alternative to NPH insulin on the basis of the assumptions used in this study.

| Intervention | Insulin glargine | | | NPH insulin | | | Difference insulin glargine - NPH insulin | | |
|---|---|---|---|---|---|---|---|---|---|
| Baseline $HbA_{1c}$ | 10 | 9 | 8 | 10 | 9 | 8 | 10 | 9 | 8 |
| Target $HbA_{1c}$ | 8.76 | 7.76 | 6.76 | 9.2 | 8.2 | 7.2 | | | |
| Microvascular events* | 34,800 | 28,949 | 25,112 | 36,925 | 31,937 | 27,095 | -2125 | -2088 | -1943 |
| Macrovascular events* | 3664 | 3395 | 3113 | 3779 | 3513 | 3233 | -115 | -118 | -120 |
| Mortality* | 4336 | 3955 | 3567 | 4477 | 4120 | 3741 | -140 | -165 | -174 |
| Total cost per patient (CHF) | 70,447 | 59,479 | 50,247 | 71,989 | 60,399 | 50,407 | -1542 | -920 | -160 |

*Per 10,000 patients over 10 years

*Supported by: Aventis.*

## 958

**Economic evaluation of the use of insulin glargine compared with NPH insulin in patients with Type 2 diabetes in Spain**
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**Background and aims:** All new pharmaceutical products must meet acceptable thresholds of cost-effectivness set by each country in order to be approved for use in general practice. The aim of this study was to compare the cost-effectiveness of insulin glargine (LANTUS®; GLAR) and NPH insulin (NPH) in patients with Type 2 diabetes in Spain.
**Materials and methods:** A retrospective cost-utility analysis was carried out using a deterministic diabetes model based on the Spanish National Health System. The model utilizes a hypothetical patient population whose baseline characteristics are defined using results from the United Kingdom Prospective Diabetes Study (UKPDS), and simulates the course of their diabetes over 10 years. Event probabilities, resource utilization and direct costs were obtained from the UKPDS, comparative clinical trials and a Spanish Health Cost database. Health utility values (quality adjusted life years [QALYs]) were obtained from the Cost of Diabetes in Europe – Type 2 (CODE-2) study. Univariant and multivariant sensitivity analyses of the base case were performed.
**Results:** Compared with NPH, the average additional health utilities per GLAR-treated patient were 0.238 and 0.254 QALYs, with and without discount, respectively. The additional cost per QALY obtained with GLAR versus NPH was €9243 and €10,969, with and without discounts, respectively (2002 prices). These costs are well below the acceptable cost-effectiveness threshold in Spain of €30,000 per QALY gained. The sensitivity analyses confirmed the robustness of the base case analysis, with costs per QALY of €2000–11,000, except when the impact of the fear of hypoglycaemia was not considered (giving costs per QALY greater than €30,000).
**Conclusion:** According to this model, GLAR is more effective than NPH, with a lower incidence of severe hypoglycaemia and with possible improvements in $HbA_{1c}$. Therefore, GLAR is a cost-effective alternative to NPH in patients with Type 2 diabetes; more QALYs are gained and costs reduced due to fewer long-term complications compared with NPH.
*Supported by: Aventis.*

## 959

**A comparison of the cost-effectiveness of basal-bolus therapy of Type 1 diabetes using insulin detemir + insulin aspart versus human insulin-based regimens**
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**Background and aims:** Poor glycemic control is associated with increased risk of complications in type 1 diabetes. A recent clinical trial demonstrated that basal/bolus treatment of type 1 diabetes with insulin detemir+insulin aspart (IDet/IAsp) improved HbA1 c (0.22%-points lower after 18 weeks), reduced risk of hypoglycemic events (by 21%), and decreasing body mass index (BMI) (–0.33 kg/m-2) in comparison to NPH insulin + human soluble insulin (NPH/HSI). A method was sought to link these short-term outcomes to long-term complication rates and associated costs, comparing these regimens in a United Kingdom (UK) setting.
**Materials and methods:** A validated model projected long-term complications, improvements in Quality-Adjusted Life Years (QALY), long-term costs, and cost-effectiveness for IDet/IAsp vs. NPH/HSI. Standard Markov modeling was used to describe incidence and progression of complications (cardiovascular disease, neuropathy, renal and eye disease). Probabilities of complications and HbA1 c-dependent adjustments were derived from the DCCT, UKPDS, and WESDR studies. Clinical input was taken from a 26 week multi-center, multinational, open-labeled, parallel-group comparison phase III trial in type 1 patients. Costs of treating complications were retrieved from published sources. Direct costs of diabetes complications and treatment with IDet/IAsp or NPH/HSI were projected over patients' lifetimes from a UK National Health Service perspective. Costs were discounted at 6 % p.a., QALYs at 1.5 %.
**Results:** Improved glycemic control, decreased hypo events and BMI with IDet/IAsp vs. NPH/HSI led to decreased diabetes-related complications, with a subsequent increase in QALY of 0.20 years, increased total lifetime costs/patient of UK£1435, and an incremental cost-effectiveness ratio of UK£7234 per QALY gained.
**Conclusion:** The decreased long-term complications, higher quality-adjusted life expectancy, and reduced costs of complications resulted in a cost-effectiveness ratio within the range generally considered to represent excellent value for money by UK and international standards.
*Supported by: Novo Nordisk A/S and Novo Nordisk Limited UK*

## 960

**Cost-effectiveness of detemir-based basal/bolus therapy versus NPH-based basal/-bolus therapy for Type 1 diabetes in a UK setting. An economic evaluation based on a meta-analysis of four clinical trials**

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**Background and aims:** A meta-analysis of results from 4 clinical trials in type 1 diabetes patients showed that insulin detemir (IDet)-based basal/bolus treatment of type 1 diabetes led to improved HbA1 c (0.15%-points lower), reduced risk of all hypoglycemic events (by 7%), and decreased body weight (0.77 kg) compared to NPH insulin-based basal/bolus therapy in type 1 patients.
**Materials and methods:** A published, validated, peer-reviewed Markov simulation model (the CORE Diabetes Model) projected short-term clinical results (changes in HbA1 c, BMI and hypo rates) obtained from a meta-analysis of four clinical trials to long-term incidence of complications (cardiovascular disease, neuropathy, renal and eye disease), improvements in Quality-Adjusted Life Years (QALY), long-term costs, and the cost-effectiveness for detemir combinations vs. NPH combinations used in type 1 diabetes patients. Probabilities of complications and HbA1 c-dependent adjustments were derived from the DCCT, UKPDS, WESDR and other published studies. Costs of treating complications in the UK were retrieved from published sources. Total direct costs (complications+treatment costs) for each treatment arm were projected over patients' lifetimes from a UK National Heath Service perspective. Costs were discounted at 6% p.a., QALYs at 1.5%.
**Results:** Improved glycemic control, decreased hypogly-cemic events and BMI with IDet-based basal/bolus therapy led to decreased diabetes-related complications, an increase in QALY of 0.12 years, increased total lifetime costs/patient of UK£1,277, and a cost-effectiveness ratio of UK £ 10,747 / QALY gained.
**Conclusion:** Short term improvements seen with IDet combinations vs. NPH combinations were projected to lead to decreased diabetes complications, improvements in QALYs, and reductions in costs of complications, which partially offset the additional costs of detemir, leading to a cost-effectiveness ratio which fell within the range considered to represent excellent value for money.
*Supported by: Novo Nordisk A/S Bagsværd and Novo Nordisk Limited, UK*

## 961

**Economic analysis of insulin glargine compared with NPH insulin in the treatment of patients with Type 1 diabetes in Spain**

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[1]HERO Consulting, Health Economics and Research of Outcomes, Madrid, Spain, [2]Aventis Pharma, Madrid, Spain, [3]Aventis Pharma, Bridgewater, NJ, USA, [4]University of Las Palmas de Gran Canaria, Spain.

**Background and aims:** All new pharmaceutical products must meet acceptable thresholds of cost-effectiveness set by each country in order to be approved for use in general practice. The aim of this study was to compare the cost-effectiveness of insulin glargine (LANTUS®; GLAR) and NPH insulin (NPH) in patients with Type 1 diabetes in Spain.
**Materials and methods:** A retrospective cost-utility analysis was carried out using a deterministic diabetes model based on the Spanish National Health System. The model uses a hypothetical patient population whose baseline characteristics are defined using results from the Diabetes Control and Complications Trial (DCCT) and simulates the long-term outcomes of their diabetes over 9 years. Event probabilities, health utilities, resource utilization and direct costs used in the model were obtained from the DCCT, comparative clinical trials and a Spanish Health Cost database. Univariant and multivariant sensitivity analyses of the base case were performed.
**Results:** The average additional health utilities per patient with GLAR versus NPH, with and without discounts, were 0.754 and 0.799 quality adjusted life years (QALY), respectively. The additional cost per QALY gained with GLAR versus NPH was calculated as € 2340 and € 2755, with and without discounts, respectively (2002 prices). These values are well below the acceptable threshold in Spain of € 30,000 per QALY gained. The sensitivity analyses confirmed the robustness of the base case, except when the impact of the fear of severe hypoglycaemia upon health utilities was not considered. However, even in this scenario, GLAR was more effective than NPH at a cost per QALY of €13,000–90,000.
**Conclusion:** According to this model, GLAR is more effective than NPH, with a lower incidence of severe hypoglycaemia, improved $HbA_{1c}$ and a reduction in long-term diabetic complications. This analysis also shows that GLAR is a cost-effective alternative to NPH in patients with Type 1 diabetes; more QALYs are gained with reduced costs due to the reduction of long-term complications compared with NPH.
*This study was supported by Aventis.*

## 962

**The association between obesity (BMI) and health-related utility in subjects with Type 1 and Type 2 diabetes**

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**Background and aims:** Obesity, the primary cause of type 2 diabetes, is reaching epidemic proportions in some countries. Insulin treatment also directly increases obesity. The purpose of this study was to characterise the relationship between obesity and health-related utility in type 1 (T1DM) and type 2 diabetes (T2DM).
**Materials and methods:** Data were taken from the Health Outcomes Data Repository (HODaR), which includes medical histories, biochemistry, health-related utility, height, weight and demographic data for a large population in the UK. The data used here were derived from 14,775 subjects (1.2% T1DM; 7.1% T2DM) sampled from hospital inpatients and outpatients. Obesity was measured using body mass index (BMI) and utility values were generated using the EQ-5D, a measure widely used to measure benefit in economic analyses.
**Results:** There was a linear and inverse association between BMI and utility at BMI >21 kg/m² (table). There was a systematic difference in utility between people without diabetes and T2DM of around 0.11 utility units. The gradient was the same in people with diabetes, T1DM and T2DM; however, the intercept changed. The gradient could be estimated using the equation: utility= -.0105xBMI+intercept.
**Conclusion:** There was an inverse relationship between obesity and health utility in people with type 1 and type 2 diabetes. Although there existed wide variability, there was definite structure within these data, this association being highly robust in extensive analysis. The absolute impact of obesity on quality of life can only be estimated after standardization for confounding factors.

Health-related utility versus BMI

| | Non-diabetes | | T1DM | | T2DM | |
|---|---|---|---|---|---|---|
| BMI | Mean | SD | Mean | SD | Mean | SD |
| 22 | 0.73 | 0.29 | 0.79 | 0.17 | 0.60 | 0.35 |
| 24 | 0.72 | 0.29 | 0.78 | 0.29 | 0.57 | 0.32 |
| 26 | 0.69 | 0.31 | 0.82 | 0.22 | 0.53 | 0.35 |
| 28 | 0.67 | 0.32 | 0.75 | 0.34 | 0.58 | 0.34 |
| 30 | 0.65 | 0.31 | 0.58 | 0.41 | 0.55 | 0.30 |
| 32 | 0.60 | 0.35 | 0.48 | 0.30 | 0.55 | 0.33 |
| 34 | 0.56 | 0.38 | 0.58 | 0.39 | 0.47 | 0.41 |
| 36 | 0.59 | 0.35 | 0.33 | 0.26 | 0.44 | 0.30 |

# PS 91

# Monitoring of glycaemia

## 963

**Relationship between $HbA_{1c}$ and daily fluctuations of blood glucose in patients with Type 1 diabetes mellitus**

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**Background and aims:** Blood glucose (BG) concentrations in diabetes vary widely during a 24-h period and from day-to-day. The measurement of $HbA_{1c}$ is the most accepted indicator of long-term glycemic control. However, the $HbA_{1c}$ levels do not provide a measure for the magnitude or frequency of short-term fluctuations of BG, wich are particularly great in type 1 diabetes (T1DM). The goal of the present work was to reveal correlation between time of BG measurements, mean glycemia, hypoglycemic episode frequency and $HbA_{1c}$ values in patients with T1 DM.
**Materials and methods:** Totally, 84 pts with T1DM were included in the study (mean age 24 ± 4 yrs., diabetes duration 9.8 ± 5 yrs.). According to $HbA_{1c}$ indices pts were divided into 3 groups (Gr.): Gr. 1 - (n=29). $HbA_{1c}$ levels of < 7%; Gr. 2-(n=32), $HbA_{1c}$ of 7 - 8.5%; Gr. 3 - (n=23) $HbA_{1c}$ of 8.5–10%. Pts from Gr. 1 and 2 were on intensive insulin therapy, and from Gr. 3 were treated conventionally. For 4 consecutive weeks all pts performed home BG monitoring - pre-breakfast (8 am), 2-h post-breakfast, pre-lunch (2 pm), pre-dinner (7 pm) and at bedtime (11 pm) BG was measured every day.
**Results:** In Gr. 1 strong correlation between $HbA_{1c}$ (6.1 ± 0.19%) and pre-breakfast ($r=0.75$, $p<0.01$), pre-lunch ($r=0.68$, $p<0.04$), at bedtime ($r=0.81$, $p<0.001$) BG indices and hypoglycemia episode frequency ($r=-0.56$, $p<0.02$) was revealed. In Gr. 2 there was also strong correlation between $HbA_{1c}$ (8.0 ± 0.51%) and pre-breakfast ($r=0.78$, $p<0.001$), at bedtime ($r=0.81$, $p<0.001$) BG indices and mean glycemia ($r=0.65$, $p<0.002$). In Gr. 3 there was strong correlation between $HbA_{1c}$ (9.2 ± 0.2%) and pre-breakfast ($r=0.68$, $p<0.001$) pre-dinner ($r=0.83$, $p<0.001$) BG indices, and mean glycemia ($r=0.77$, $p<0.001$). Not very strong correlation between $HbA_{1c}$ and post-breakfast BG levels was observed in all groups.
**Conclusion:** In patients on intensive insulin therapy pre-breakfast and bedtime BG values are better predictors for good glycemic control, than post-breakfast, pre-lunch and/or pre-dinner ones. While indices of pre-breakfast and pre-lunch BG levels may be used as indicators of glycemic control in pts on conventional therapy.

## 964

**Reliability of using a small blood sample for glucose meter testing**

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**Background and aims:** Most glucose meters today are advertised as fast and requiring a tiny blood sample for a test. Therefore, people with diabetes who monitor their blood glucose may be inclined to apply less blood in their testing. We studied the effects of applying a small blood sample on six blood glucose-monitoring systems.
**Materials and methods:** Five of the systems (Meters A through E) are based on electrochemical technology; one system (Meter F) is based on photometric reflectance technology. Meters A, C and E use glucose oxidase in the test strip; Meters B and F use glucose dehydrogenase GDH-PQQ, and Meter D uses GDH-NAD. With each system, 20 tests with a blood sample were performed at each of 5 sample volumes, covering the ranges above and below the manufacturer's specification on minimum sample size, which are 5.0, 4.0, 2.0, 1.5, 1.0 and 0.6 microliters for Meters A, B, C, D, E and F, respectively. Blood was applied to the test strips using fixed-volume positive-displacement pipettes. All test results were compared to the mean glucose value obtained at 5 microliters (the control condition). A result was deemed inaccurate if it differed more than 20% from the mean value of the control condition.
**Results:** When insufficient blood was applied, Meters A, C and F gave some falsely low results with maximum biases of -96%, -92% and -33%, respectively, when compared to the control condition. Meter B gave some falsely low results as well as some falsely high results, with maximum biases of -87% to +34%. Meters E immediately gave an error message and wasted the test strip in 100% of the tests when insufficient blood was applied. Meter D did not start any test when insufficient blood was applied; adding a second drop of blood within 30 seconds of the first drop produced an accurate result.
**Conclusion:** These findings indicate that, when users apply less blood than what the test strips are designed for, some glucose monitoring systems can give erroneous results and some systems immediately give an error message but the test strip has been wasted. This study also demonstrates that one can minimize inaccurate results and wasted test strips due to insufficient blood if the test strip has the proper design, as shown by the Meter D test strip, which has an effective fill-trigger electrode for electronic detection of sample volume.

## 965

**Are the low-cost, blood-glucose monitoring systems reliable?**

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**Background and aims:** Healthcare providers and insurance companies consider cost when selecting a blood glucose-monitoring system for the patients. We compared a low-cost system (TrueTrack Smart System, Home Diagnostics, Inc.) and a regular-cost system (Precision Xceed, Abbott Diabetes Care) in their ability to maintain accuracy in the presence of 2 interfering substances - paracetamol (acetaminophen) and uric acid. Paracetamol is a common drug for pain relief; uric acid is present in blood at elevated levels in gout and renal failure, a long-term complication of uncontrolled diabetes.
**Materials and methods:** Venous blood samples were collected before breakfast from healthy subjects who had not taken any medications for at least 24 h before blood collection. Within 2 h after collection, the blood samples had glucose concentrations of approximately 60 mg / dL (3.3 mmol / L) and were used in the studies. In the dose-response study, aliquots of the venous blood sample were supplemented to varying concentrations of paracetamol (up to 15 mg/dL or 993 µmol/L) or uric acid (up to 20 mg/dL or 1.18 mmol/L), then each aliquot was tested 10 times with 2 glucose monitoring systems based on electrochemical technology. The TrueTrack Smart uses glucose oxidase on the test strip; the Xceed uses glucose dehydrogenase (GDH-NAD). In the additive-effect study, paracetamol (approximately 4 mg/dL or 265 µmol/L) and uric acid (final concentration in plasma: 16 mg/dL or 0.94 mmol/L), alone and in combination, were added to aliquots of the venous blood sample and then each aliquot was tested 10 times with the 2 monitoring systems. Results were compared to the control blood aliquot with no substance added. With therapeutic doses, blood paracetamol levels up to 4 mg/dL (265 µmol/L) have been reported. Blood uric acid levels above 10 mg/dL (0.59 mmol/L) are common in renal failure.
**Results:** In the dose-response study, results of the TrueTrack Smart increased as a function of the concentrations of paracetamol and uric acid, with maximum biases of 93% and 46%, respectively. Results of the Xceed did not show any changes of clinical significance (maximum biases <5%). In the additive-effect study, adding paracetamol, uric acid, and their combination produced significant biases ($p<0.0001$) of 25%, 39% and 60%, respectively with the TrueTrack Smart, which reported falsely euglycemic values of 77, 85 and 97 mg/dL (4.3, 4.7 and 5.4 mmol/L), respectively on the hypoglycemic blood sample (61 mg/dL; 3.4 mmol/L). These substances, alone or combined, did not cause any significant changes ($p>0.1$; all biases <2%) in the results of the Xceed system.
**Conclusion:** We found that the TrueTrack Smart system was very sensitive to interference by paracetamol and uric acid, and gave falsely higher glucose results. The Xceed system maintained accuracy when challenged in this study. These findings raise a concern that the quality of results may be compromised in the low-cost glucose meters. Healthcare providers and patients need to be cautious when selection a glucose monitoring system.

## 966

**Evaluation of metabolic control in gestational diabetic women (GDM) by the continuous glucose monitoring system (CGMS)**

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**Background and aims:** Evaluation of blood glucose concentration in a group of women with gestational diabetes mellitus (GDM), under the care of Endocrine Clinic of the RI PMMH Lodz, Poland, by the Continuous Glucose Monitoring System (CGMS).
**Materials and methods:** Seven (7) diet-controlled women with gestational diabetes (G1), five (5) diet- and insulin-controlled GDM women (G2) and

seven (7) healthy, pregnant women (N) were included in the study. GDM diagnostics was performed between the 24th and the 28th week of gestation and was based on the results of an oral glucose tolerance test (75 OGTT), according to the WHO. The treatment was adjusted according to self-monitoring of blood glucose (SMBG), using an Accu-check Active- Roche Glucometer. The self-monitoring was performed 4 times a day aiming for fasting blood glucose values < 90 mg/dL and postprandial (2 hours after meal) <120 mg/dL. Then, the patients were submitted to a 72-h period of the Continuous Glucose Monitoring System (Medtronic-Minimed System, 5-minute readings), which provides continuous blood glucose profile and allows for evaluation of metabolic compensation.

**Results:** The three examined groups N, G1, G2, disclosed no difference ($p>0.05$) for individual parameters, measured with CGMS with regard to: mean 24-hour glycaemia (85 ± 6; 87 ± 14; 91 ± 18 mg/dl), fasting glucose level (79 ± 13; 88 ± 17; 82 ± 9 mg/dl), postprandial glucose level (96 ± 11; 97 ± 27; 105 ± 22 mg/dl), the mean glucose level during night (77 ± 6; 71 ± 11; 75 ± 23 mg/dl), and the area under glycaemia curve (281 ± 37; 315 ± 35; 310 ± 59), respectively. Neither was there any difference in the total duration time of glycaemia below 60 mg/dl (317 ± 229; 300 ± 264; 370 ± 324 min.) nor the duration of glycaemia of more than 120 mg/dl (259 ± 400; 225 ± 229; 394 ± 539 min.) in group N, G1 and G2, respectively ($p>0.05$). However, when using CGMS, in comparison to SMBG, a wider range of glycaemia was observed in all the examined groups. For the healthy, pregnant women N: 41–194 *vs.* 61–151 mg/dl, for G1: 40–244 vs. 40–180 mg/dl, for G2: 40–173 *vs.* 50–157 mg/dl were observed.

**Conclusion:** The therapy, based on the self-monitoring of blood glucose levels, when applied to the group of gestational diabetic women, brought the said glucose levels under an effective control, with mean outcome values similar to those observed in the group of normal pregnant women. However, by the means of CGMS we detected long, asymptomatic periods of high and low glycaemia, both in the diabetic and in the unaffected pregnant women.

## 967

### Association between maternal glucose during delivery and postnatal glucose adaptation of the infant

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**Background and aims:** Early postnatal hypoglycaemia is a frequent observation in newborn infants of mothers with diabetes, requiring postnatal feeding and occasionally intravenous glucose treatment. In this pilot study we have used the continuous glucose monitoring system (CGMS) to obtain a detailed picture of the maternal glucose control during delivery which is supposed to be related to the postnatal blood glucose adaptation of the infant.

**Materials and methods:** We have so far included 6 pregnant mothers with insulin-treated diabetes mellitus (5 IDDM and 1 GDM). Their age was 27 – 33 years and normal delivery was obtained after 37 – 39 weeks of gestation. During delivery guidelines for control was to check plasma glucose every hour and to inject subcutaneous rapid-acting insulin if plasma glucose exceeded 7 mmol/L. In all mothers a CGMS registration was initiated at least 2 hours before delivery (CGMS, Medtronic, USA). From the CGMS registration we calculated the mean glucose concentration and the area under the curve (AUC) glucose concentration during 60 and 120 minutes before delivery.

All infants were transferred to the neonatal care unit for blood glucose measurements at 1 and 3 hours of age and thereafter every third hour during the first 24 hours of life. Early oral feeding with formula was given at two hours of age and thereafter every third hour. I.v. glucose was given if repeated blood glucose was below 1.5 mmol/L.

**Results:** Maternal glucose concentrations 60 and 120 minutes before delivery and postnatal infant blood glucose concentrations (mmol/L) at 1 hour are presented in the table.

| | AUC 0–120 | Mean 0–120 | AUC 0–60 | Mean 0–60 | Weight (g) | pH | B-glucose 1 h | i.v glucose |
|---|---|---|---|---|---|---|---|---|
| 1 | 1033 | 8.6 ± 2.9 | 369 | 6.2 ± 1.0 | 4035 | 7.2 | 1.0 | yes |
| 2 | 841 | 7.1 ± 1.7 | 501 | 8.4 ± 1.3 | 4525 | 7.0 | 0.7 | yes |
| 3 | 707 | 5.9 ± 0.7 | 389 | 6.5 ± 0.3 | 3700 | 7.4 | 4.1 | no |
| 4 | 672 | 5.6 ± 1.1 | 286 | 4.8 ± 0.1 | 5020 | 7.4 | 2.8 | no |
| 5 | 627 | 5.3 ± 1.0 | 329 | 5.5 ± 1.3 | 3330 | 7.2 | 2.7 | no |
| 6 | 614 | 5.2 ± 0.7 | 299 | 5.0 ± 0.4 | 4755 | 7.4 | 2.9 | no |

**Conclusion:** In this pilot study, using CGMS registrations, there seems to be an association between maternal glucose concentrations 120 minutes before delivery and the postnatal glucose adaptation and need for i.v. glucose treatment of the infants. Maternal euglycaemia during delivery might reduce the occurrence of postnatal hypoglycaemia in the infants.

# PS 92

# I.T. and telemedicine

## 968

**Telemedicine system for monitoring diabetes during pregnancy: a preliminary study**

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**Background and aims:** Pregnancy complicated by diabetes is an interesting field for telemedicineapplication: due to necessity of tight glicemic monitoring, frequent contacts with diabetologist and difficulty to reach specialized centers (distance and/or pregnant forced to bed). We estimated efficacy, users acceptance and impact on quality of life (DQL) of telemedicine in pregnant women followed in 12 Italian Diabetic Clinics.
**Materials and methods:**120 patients (100 GDM, 20 type 1 diabetes) were randomized to receive diabetes monitoring by telemedicine (T) or traditional glucose monitoring (C); T GDM patients were educated to use telemedicine 2 weeks after diagnosis, type 1 at 1st visit after conception; each subject was asked to complete the DQL scale (CES-D, Stress, Distress, SF-36). GDM women used telemedicine for 2 months, type 1 for all pregnancy. At the end of pregnancy they completed DQL scale and a questionaire for treatment satisfaction. T patients come to the center with monthly frequency, the C every 2 weeks.
**Results:** Age, BMI were not different in both groups. HbA1 c did not differ significantly in the 2 GDM groups; T type 1 showed HBA1 c reduction (C= 7.0 ± 0.1% vs 6.8 ± 0.4%, T= 7.5 ± 1.4% vs 6.8 ± 1%). Maternal and fetal outcome did not differ in the 2 groups. T GDM patients had 9 ± 5 conctacts and the answers of the doctors were 5 ± 4,T type 1 had 18 ± 13 contacts and the answers were 13 ± 10. About system, 15,7% of patients had occasional problems with glucobeep and 3,9% had frequent problems. The 32,7% had occasional problems with connection to the server and/or to green number. Telephone problems were referred sporadically in 32,7%, frequently in 9,6% of cases. Vocal message was suitable for 77,5% of shipment cases and 97,8% of the receipt cases. Medical message was considered comprehensible in 81% of the cases; 84% considered suitable 1 transmission a week, 10% 2 times. 90% of the women considered the system good, 10% sufficient. 94% would continue to use it. 47% of the women considered telemedicine a therapeutic system at home, 45% a therapeutic system at home and educational instrument ; 4% of women considered it a system useful for metabolic emergency.
**Conclusion:** Patient satisfaction was high in the telemedicine group. This pilot study show the flexibility of the system in clinical routine use and its potential benefits for diabetics care in pregnant women; it is important for health saving that a not different outcome was achieved in pregnant women using it with respect to controls with a less number of specialist visits. It offered a better physician-patient communication procedure. DQL analysis in both groups of patients is ongoing.

## 969

**VIE-DIAB: Telemedical support Programme for adolescents with Type 1 diabetes mellitus**

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**Background and aims:** Acceptable metabolic control is difficult to achieve in diabetes during puberty. The aim of the study was to test the feasibility of telemedicine support in adolescent patients with diabetes using mobile phones and Internet services and to test whether an increasing communication between patients and diabetologists by this approach has a positive influence on glycaemic control.
**Materials and methods:** The telemedical support system VIE-DIAB integrates data collection, visualization, and recommendation for handling by using mobile phone and Internet services. Its core is a module, which visualizes a summary of the patient's diary data of the last 4 weeks. A feedback from the diabetologist to the patient is given once a week.
Inclusion criteria for the study were a diabetes duration > 1 year, chronological age > 12 years, HbA1 c > 8 % and willingness to participate in the study.
36 adolescents (m=20, f=16) with diabetes mellitus type1 took part in the randomised cross over-study. For 3 months they documented their self-control measurements in a standard diabetes diary as usual and for another 3 months they additionally sent their data daily by SMS to the central server using the VIE-DIAB system. Group1 started with the VIE-DIAB system, group2 began with the diaries only, and after 3 months they switch to the other procedure.
At the end of the study a questionnaire on patients experiences and satisfaction was applied.
**Results:** The mean age of the participants was 15,3 ± 1,9 yrs, the mean diabetes duration was 6,8 ± 3,1 yrs. and the mean HbA1 c was 9,2 ± 0,9 %. In group 1 mean HbA1 c at 0, 3 and 6 months was: 9,2 ± 0,9, 9,1 ± 1,3 and 9,4 1.4 % respectively, while in group 2 the HbA1 c values varied between 9,3 ± 1,0, 9,7 ± 0,9 and 9,4 ± 1,4 % respectively.
There was a trend to lower HbA1 c values after the three-month period on telemedical support. During the study period 14 patients showed a HbA1 c reduction of > 0.5 % using telemedical support compared to only 4 participants using standard diabetes diary documentation ( $p < 0.05$). We did not observe any differences concerning DKA or severe hypoglycemia. 22 (of 36) participants complained of technical problems during the study, despite these problems 21 participants considered the VIE-DIAB helpful for the everyday management and 20 wanted to continue.
**Conclusion:** The telemedical support VIE-DIAB proved to be feasible in a group of adolescents with diabetes, although there were several technical problems. It might be an option to support diabetes-patients in improving glycaemic control.

## 970

**Outcome and efficiency of restoration of metabolic control of insulin dependent diabetics by telemonitoring**

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**Background and aims:** Modern information technology, meters with interfaces, patient friendly software for data input and visualisation and the spreading of internet and email allows easy sampling, storing and safe transmission of metabolic data of diabetic patients. In a previous study we have shown that telemonitoring in insulin requiring patients can save time and costs with the same outcome as in conventional care. The aim of this study was twofold: (1) to check if telecare can reduce the length of stay in hospital after metabolic derailment (2) to test a novel email-based data transmission method.
**Materials and methods:** 55 Patients with insulin requiring diabetes (age 37,7 ± 11,5 yrs) diabetes duration 10,4 ± 9,5 yrs) with a severe problem of metabolic control were assigned to a group with telemonitoring. This group received a structured diabetes education, glycemic control was restored in part in hospital and the patient was released as soon as possible to be monitored by telemedicine. All patients were instructed into the system in person and signed a consent form approval. Weekly, bi-weekly or monthly telephone consultations with a diabetologist in our diabetes centre were performed soon after patients send their data. These included glucose profiles, insulin doses and carbohydrate contents of meals. Two systems were used: AccuCheck-CamitPro (Roche Diagnostics, Mannheim Germany) with AccuLink-modem connected to an ordinary telephone line and DIABASS with glucoNet (Mediaspects, Konstanz Germany) with email transfer of an encrypted (128 bit-DES) data-file. 60 patients (age 39,6 ± 12,6 yrs, diabetes duration 13,0 ± 9,9 yrs) with similar metabolic problems were assigned to a group with conventional treatment (education, restoration of metabolic control to near-normal glycemia in hospital and release to ambulatory treatment with their private practitioners or diabetologists). Both groups were reevaluated one year after hospital release.
**Results:** On average 6.3 ± 4,7 consultations with an average duration of 15.1 ± 10,4 min were neccessary in order to achieve the target. 19 patients did not complete the program until the final goal was reached.HbA1 c dropped from 8,6 ± 2,0 % to 7,0 ± 0,8 % in the telecare group and from 8,3 ± 1,5 % to 7,9 ± 1,2 % ( reevaluation after one year). Length of hospital stay was 7,1 ± 2,7 days in the telecare and 12,3 ± 5,6 in the conventional group. In a structured questionaire, 93 % would again seek for advise by telecare in the future, if another problem occurs.
**Conclusion:** Telemonitoring with frequent telefon visits has been evaluated in a number of studies, some comprising a randomised control group with

conventional care. In our non-randomised study, the telemonitoring approach resulted in a shorter hospitalisation period, the outcome of telemonitoring was better than conventional care regarding the glycated hemoglobin. In conjunction with our earlier results of saving costs and time due to less travel expenses and for not working, telecare in diabetes ist efficient particularly in a health care system, that is currently adopting diagnosis related groups (DRG's) such as Germany. It can now be considered as a method of routine management of diabetes. Minor improvements in the data presenting software and the transmission method are necessary. Non adherence of patients until the final goal is achieved may be a problem, that can in part be solved by hand-held devices, that can store the complete data set.
*Supported by Bavarian Ministry of Social Affairs*

## 971

**Feasibility of a smart phone based data service for intensified insulin therapy in patients with Type 1 diabetes mellitus**
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**Background and aims:** Metabolic control in type 1 diabetic patients (D-1) on intensified insulin therapy depends on the compliance regarding blood glucose (BG) monitoring, the adherence to the algorithms to correctly administer insulin and on the proper documentation of the respective parameters for evaluation by the physician.
**Materials and methods:** The aim of this pilot study was to determine the level of acceptance, stability and error rate of a wireless and mobile data service for transmission of diabetes related health parameters such as insulin doses and BG in D-1 to a Diabetes Clinic based on cellular phone technology. 8 (2 female, age 36.1 ± 11.4 (mean ± SD) y) D-1 treated by ICT were asked to enter and transmit all BG values, insulin doses (ID) and hypoglycemic episodes (HYPO) using a smart phone based diabetes diary over a period of three months. If less than 3 BG values were submitted per day, an automated reminder message was sent to the patient via short message service (SMS).
**Results:** The total number of received values was 10053 (1257 ± 351 per patient), BG: 3341 (418 ± 127), ID: 3871 (484 ± 147), HYPO: 239 (30 ± 25; 0.3 ± 0.1 per patient/day). 94.6% of the transmission attempts were successful resulting in an average of 14 transmitted values per patient and day. The number of wrong input values, as reported by the patients, including date/time failures was 143. This resulted in an error rate of 1.4 %. About 8.6 % of the values were sent via desktop computer mainly by two patients using the internet access at their workplace. Average BG during the initial and final 14 days was 138.5 ± 63.7 vs. 137.1 ± 69.2 mg/dl, HbA1 c 7.7 ± 0.4% vs. 7.6 ± 0.4%, respectively.
**Conclusion:** Ubiquitous availability and the easy-to-use menu were well perceived by the patients. These results indicate a high success rate for data transmission attempts and patient acceptance. Further studies should determine whether this method results in an improved compliance and subsequently better metabolic control.

## 972

**Attitudes of young people with diabetes to an internet-based virtual clinic**
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**Background and aims:** Many young people with diabetes fail to attend routine appointments at clinic. This may be part of the cause for the reduction of adherence to treatment regimes in this age group, which can result in complications or the need for hospital admissions, in later life. Thus, there is a pressing need to look at innovative ways which engage directly with young people, in order to reduce the effects of non-attendance at clinics.
The aim of this study was to assess the feasibility of developing a 'virtual clinic' using the internet. The proposed content of the site would be based on self-efficacy theory, aiming to develop confidence in self-management of diabetes. This would include 24-hour access to records, information and advice, and a 'chat room' for peer support.
**Materials and methods:** A questionnaire was designed covering: existing access and use of the internet; attitudes towards a virtual clinic; what facilities it should include; likelihood that they would use such a service. The questionnaire was delivered to 72 patients registered at a clinic, aged between 11 and 16, in the period December 03 to February 04. Replies were made using a pre-paid envelope.
**Results:** 39 (54%) responses were received (64% male, ages 11 to 16, mean 13). Duration of diabetes ranged from less than 1 year to 12 years. 37 (95%) used the internet at home and 35 (90%) used it both at home and at school. Other places were friends' home, internet café, or public library. Mobile phone access was little used. The internet was used more than twice a week by 85% and most often for schoolwork and games.
A positive attitude to a potential virtual clinic was reported by 95% of responders. The items rated as most useful were quick and easy access to up-to-date information, the opportunity to ask an expert, good graphics, easy navigation, and interactivity. Responses to open questions indicated that 24-hour access and anonymity for asking questions were also valued.
**Conclusion:** An internet-based, virtual clinic for young people with diabetes would be both acceptable and accessible to young people. Quick and easy access to reliable information, opportunities to ask questions of experts and to learn from each other were all valued highly. These aspects all are supported by the theory of self-efficacy as effective methods to increase confidence in a person's ability to self-manage their diabetes. A virtual clinic is a possible method for effective health care delivery to young people with diabetes.
*Supported by: Warwick West Midlands Primary Care Research*

## 973

**The Internet as an effective tool in the prevention and therapeutical reinforcement of diabetes mellitus - The national information system www.diabetes-deutschland.de**
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**Background and aims:** The website www.diabetes-deutschland.de is a national information system promoted by the Federal Ministry of Health and Social Affairs in Germany. It offers information on diabetes to patients, the general public and specialists. Recent studies indicated the growing interest of the elderly population in the Internet. Since the incidence of Type 2 continues to be high in this group, we aimed at studying the degree to which this group can be reached through our website and how Internet can be employed in the prevention and therapeutic management of Type 2 diabetes.
**Material and methods:** We analysed the access statistics of www.diabetes-deutschland.de monthly. An online questionnaire was available for 43 days and served as an intercept survey. Participants were invited through pop-up windows, online news and e-mail newsletter. The scope of the survey qestions varied according to the user categories.
**Results:** The website recorded 21,584 visitors and 196,772 page-impressions in December 2003, thus showing a significant increase of 250% within one year. We collected 1,132 data sets from 1,600 registered participants: 93.1% from Germany, 6.9% from other German-speaking countries (Austria 1.7%, Switzerland 3.3%), 1.9% from non-German-speaking nations.
There were 67.5% of people with diabetes, 17.2% professionals; 10.3% combined private and professional interest. Of the people with diabetes, 38.8% had Type 1 and 55.1% Type 2 diabetes. The non-diabetics sought information on behalf of Type 1 (41.1%) and Type 2 diabetics (47.8%). Type 2 predominated (70–75%) in all groups >50 years, while Type 1 was mainly found among those <39 years (30–39 yrs:76.8%, 20–29 yrs:84.8%, 18–19 yrs:90%). There was even distribution in the 40-to-49 age group: 47.5% Type 1 and 44.0% Type 2.
675 of the participants had diabetes, 217 sought information on behalf of others, i.e., for children (27.2%), parents (22.1%), life-partner (19.8%), friends (10.1%), other relatives (7.4%), or other (13.4%). The majority sought information for themselves (75.4%). The knowledge gained was valuable to specialists in their professional activities (77.6%); 76.2% intended to recommend the website. Most participants (69.2%) felt the website had improved their knowledge, 65,0% used it as a reference source, and 62.2% accessed the online news. Among diabetics and those involved with them, 86.3% claimed they were more knowledgeable after access, 44.0% felt better prepared for a doctor's appointment, and 40.3% intended to improve their daily routine.
**Conclusions:** Our results show a definite need for information about diabetes, felt by diabetic patients, their relatives, and professional users. We confirm other published results, in that older population groups (corresponding to our users with Type 2 diabetes) are interested in the Internet as a medium of information. Our preliminary assessment indicates the potential value of the website in the prevention of diabetes and in the reinforcement of therapeutical strategies. For Germans living abroad, this website offers easy access to valuable information in their mother-tongue. Our findings also show potential but also a necessity of continous optimisation, up-to-dateness and service.

# PS 93

# Screening and insulin sensitivity in gestational diabetes

## 974

**The prognosis of gestational diabetes mellitus is improved when universal rather than selective screening is performed.**

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**Background and aims**: Very few data are available regarding the maternal and foetal prognostic impact of universal rather than selective screening for gestational diabetes mellitus (GDM). The aim of this study was to compare health benefits from selective or universal screening in women with GDM;
**Material and methods**: GDM was assessed with a 75-g oral glucose tolerance test, and defined as a fasting glucose value >5.3 mM, or a 2-hr glucose value >7.8 mM, or both. The offspring and maternal outcomes were compared in two series of women with GDM: 1) the 159 women with GDM out of 1909 consecutive women who delivered between Oct 2000 and Sept 2001 (prevalence of GDM: 8.3%); during this period screening was based on risk factors (major criteria: age, familial history of diabetes, body mass index, characteristics of previous pregnancy); 2) the 265 women with GDM out of 2111 consecutive women who delivered during the year 2002 (prevalence of GDM: 12.6%), during this period screening was universal. The women were screened between 24 and 28 weeks of gestation, or later when macrosomia, glycosuria or hypertension were observed during selective screening;
**Results**: Compared with the women with GDM diagnosed during the universal screening period, the women with GDM diagnosed during the selective screening period were older (33.6 ± 5.6 versus 31.3 ± 5.8 years, $p<10^{-3}$), had a higher pregravid body mass index (26.1 ± 5.6 versus 25.0 ± 5.5 kg/m$^2$, p=0.036) and a higher rate of previous pregnancy with GDM (17.7 versus 14.0%, p=0.022). Lower rates of macrosomia (7.5 versus 17.6%, Odds ratio 0.38 [95% confidence interval 0.21–0.71], p=0.002), of jaundice (3.0 versus 7.5%, OR 0.38 [0.15–0.96], p=0.035), of admission to the department of paediatrics (12.5 versus 23.3%, OR 0.47 [0.28–0.79], p=0.004), of anticipated delivery with prostaglandin gel (11.7 versus 18.9%, OR 0.57 [0.33–0.98], p=0.042) or oxytocic infusion (OR 0.56 [0.37–0.83], p=0.004), higher neonatal capillary glucose value (3.5 ± 0.9 versus 3.4 ± 1.0 mM, p=0.044), and shorter maternal stay at hospital after delivery (4.9 ± 2.2 versus 5.5 ± 2.4 days, p=0.04) were observed in the women with GDM diagnosed during the universal screening period than during the selective screening period;
**Conclusion**: Compared with selective screening, universal screening for GDM detects more cases and improves maternal and offspring prognosis. We assume that universal screening facilitates earlier diagnosis and care.

## 975

**Results of screening for gestational diabetes mellitus with a 75 g 2 h oral glucose tolerance test in consideration of risk factors and adverse perinatal outcomes**

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**Background and aims:** In Belarus there is a strong tendency to rise in number of deliveries in women with pregestational and gestational diabetes mellitus (GDM) – from 2 to 8% in 5 years. Different criteria are recommended for diagnosis of GDM. Our aim was to analyze the prevalence and screening procedure for GDM among pregnant women referred to our center, and to characterize these women with regard to GDM risk factors and perinatal outcomes.
**Materials and methods:** From 1016 pregnant women with carbohydrates' imbalance (center database of 5 years – 12709 pts) we extracted 632 who had GDM according to WHO criteria - group 1. GDM had been screened by step-by-step method: 2 h 75 g oral glucose tolerance test (OGTT) was conducted to the pregnant women with high GDM risk at the diagnosis of pregnancy (8–12$^{th}$ week) and, if negative, at 24–26$^{th}$ week; in other – at 24–26$^{th}$ week of pregnancy only. We analyzed the presence of risk factors (body mass index (BMI) before pregnancy > 24 kg / m$^2$, age > 25 years, familial history of diabetes mellitus (DM), obstetrician anamnesis and complications), perinatal outcomes and weight of newborns. The control group consisted of 100 healthy pregnant women extracted by random sampling.
**Results:** The prevalence of GDM in our center was 4,97% (with growth year-by-year: from 1,25 to 11,15%). Perinatal mortality was 4,2‰ (with decrease year-by-year: from 33,3 to 3,8‰). The reliable differences between group 1 and control group in age (26,75 ± 6,02 vs 25,35 ± 4,53 yrs, p<0,05), BMI (>24 kg/m$^2$ – 43,07 vs 14%, p<0,01), newborn babies weight (3528,8 vs 3375,6 g, p<0,05; in group 1 babies weight > 4000g was in 15,15% of babies, in control group – 3,03%), frequency of hydramnion (15,49 vs 2%, p<0,01) and OPH-gestosis (26,05 vs 8%, p<0,01) were revealed. Previous large newborn (>4000g) was in 10,87%, familial history of diabetes – 13,38% of women with GDM (in control group there were no such cases). Insulinotherapy was administered in 3,52%, there were women with GDM diagnosed in the first trimester of pregnancy. Other women were given diet only.
**Conclusion:** Step-by-step method of screening of GDM with 2 h 75 g OGTT in consideration of the most reliable risk factors of GDM (age>25 years; BMI before pregnancy>24 kg/m$^2$; familial history of DM; large newborn in the previous pregnancy; developing hydramnion, OPH-gestosis or "large-for-gestation age" fetus during the pregnancy) lead to improvement of early exposure of GDM by 9,3%; to better perinatal outcomes with decrease of perinatal mortality by 29,5‰ and to reduction in obstetric complications due to more effective treatment among pregnant women with GDM.

## 976

**Usefulness of assessing glycemic status in early pregnancy for the screening of gestational diabetes mellitus**

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**Background and aims:** The exact clinical significance of alteration in glucose homeostasis in early pregnancy is yet to be specifically determined. Whether abnormal glucose homeostasis that precedes the development of gestational diabetes mellitus (GDM) can be detected and defined in the first trimester needs to be answered. The present study was undertaken to explore the usefulness of early screening for the detection and prediction of GDM and to set a cut-off value of plasma glucose (if possible) to screen subjects who are at risk of developing gestational diabetes mellitus.
**Materials and methods:** A total of 246 subjects, collected from the Out-Patient various hospitals of Dhaka city, were enrolled at their first visit within 17 weeks of gestation in this descriptive longitudinal study. Out of this 246, 211 nondiabetic subjects were asked to follow-up in the early part of third trimester; till the present analysis 89 of them followed up in the second visit in the early part of third trimester till the analysis of results. Detailed history, clinical examination and anthropometry were recorded in a pre-designed questionnaire. GDM was diagnosed by Oral Glucose Tolerance Test which was performed on all subjects following the WHO guidelines. A cut-off point of 6.75 mmol/l (the lower bound of 95% confidence limit of 2 h PG load plasma glucose of the GDM subjects on first visit) was taken and the nondiabetic subjects of first visit were divided into risk group (2 h PG load plasma glucose ≥ 6.75 mmol/l) and non-risk group (2 h PG load plasma glucose <6.75 mmol/l).
**Results:** In the first visit, 156 (66%) had Normal Glucose tolerance (NGT), 5 (2%) had Impaired Fasting Glycemia (IFG), 50 (21%) had Impaired Glucose tolerance and 25 (11%) had preexisting Type 2 Diabetes Mellitus (DM). In the second visit, 68 (76%) had NGT and 21 (24%) had GDM. The fasting plasma glucose in the first visit of GDM and NGT were comparable. But, the 2 h post glucose load plasma glucose in the first visit, although within nondiabetic range, was significantly higher at second visit in GDM subjects compared to NGT (7.6 ± 2.0 vs 6.0 ± 0.9, P <0.001). In the risk group, a significantly greater proportion developed GDM compared to the non-risk group (36.6% vs 12.5%, $\chi^2$ 31.794 at 2 df, P < 0.0001). The rates of conversion to GDM from first visit were as follows: NGT- 10/65 (15%), IFG 1/1 (100%) and IGT 10/23 (43%). Conversion of IFG and IGT subjects were significant ($\chi^2$ 7.635 at 1 df, P <0.01).
**Conclusion:** Screening at early pregnancy by Oral Glucose Tolerance Test is useful to detect preexisting diabetes and the subjects at risk of developing gestational diabetes. At early pregnancy, non-diabetic subjects with the 2 h post 75 g load plasma glucose higher or equal to 6.75 mmol/l have higher probability of developing gestational diabetes.
*Supported by: Diabetic Association of Bangladesh*

## 977

**Clinical risk factors and their influence on the development of gestational diabetes mellitus**

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**Background and aims:** In different pregnant populations the rate of risk factors of gestational diabetes mellitus (GDM) and their influence on the development of disease varies significantly.
The aim of our study was to determine the occurence of predicting factors of GDM, and the value of individual risk factors on the development of GDM.
**Materials and methods:** After a fasting blood glucose measurement a standard seventy-five-gram (75 gm) two-hour oral glucose tolerance test (oGTT) screening was conducted among 3057 pregnant women between 1 November 2000 and 30 January 2003 at the I.st Department of Obstetrics and Gynaecology Semmelweis University of Medicine Budapest. The received data were analized in our retrospective study.
A diagnosis of GDM was made when the 120 min. glucose concenrtation met or exceeded the 7.8 mmol/l. The gestational age in screened population was 26.7 ± 7.1 week in the average. The used questionnaire contained questions referring the obstetrical hystory and the traditional risk factors. The abstracted variables for each pregnancy included maternal weight (BMI>30 kg/m), maternal age (≥35 yrs), positive family history of diabetes, GDM during previous pregnancy, macrosomia during the actual pregnancy, polyhydraminos (amnionic fluid index >24 cm), prior macrosomia (birth weight ≥4000 gm), multiple pregnancy and chronic hypertension.
As statistical method logistic regression analysis was performed using SPSS for Windows version 11.0 (SPSS Inc. Chicago, Ill.). The p value of <.05 was defined as significant.
**Results:** The incidence of GDM was 10.6% in the examined population.
As the five most frequent risk factors the high maternal BMI 24.1 %, high maternal age 11.1%, polyhydramnios 5.1%, prior macrosomia 4.9% and the positive history of diabetes mellitus in family 4,5% were found. All but chronic hypertension the examined risk factors have increased significantly the occurence of GDM.
Among the risk factors the maternal obesity(OR:1.71, $p<0.00001$), prior macrosomia (OR : 1.73, $p < 0.03$), prior multiple pregnancy (OR : 1.51, $p < 0.0001$) and the ultrasound diagnosed polyhydramnios (OR : 1.85, $p < 0.0001$) were found as having the most considerable effect on GDM.
**Conclusion:** In the examined pregnant population a high incidence of GDM was found. The presence of risk factors produced an almost doubled occurence of GDM.

## 978

**A population based study (G-DISS) of diagnosis of gestational diabetes and pregnancy outcome. Importance of fasting blood glucose**

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**Background and aims:** Different methods are used for screening and diagnosis of gestational diabetes (GDM). Our aim was to characterize women with GDM and pregnancy outcome in a population based study, when diagnosed by a 75g oral glucose tolerance test (OGTT) with a 2 h capillary blood glucose ≥9.0 mmol/l.
**Materials and methods:** From 1995 to 1999, prospective registration of GDM was performed in whole Sweden (G-DISS). Country of birth, height, prepregnancy weight, calculated day for delivery, indication for OGTT, fasting and 2 hr blood glucose were reported. Data about delivery for women with GDM and 2 matched controls were obtained from the Swedish Medical Birth Register
**Results:** Criteria for GDM were fulfilled in 2085 incident cases. Women with GDM were older, shorter, had higher BMI and were twice as often born in non-European countries in comparison with all women who delivered in Sweden. Average birth weight in GDM was 3683 ± 616 g (mean ± SD) with 8.6 % ≥ 4500 g. In women without GDM (n=3638) birth weight was 3542 ± 96 g with 4.1% ≥ 4500 g. Noteworty the increased birth weight in GDM was related to fasting blood glucose and not to the level of 2 h blood glucose above 9 mmol/l.
**Conclusion:** Women with GDM are shorter, older, have higher BMI and are more often of non-European origin than the background population. The birth weight in GDM pregnancies is increased and related to fasting blood glucose at diagnosis. Our results suggest that it is important to also consider fasting blood glucose at diagnosis of GDM.
*Supported by: Swedish Diabetes Association*

## 979

**Indexes of insulin sensitivity and B-cell function in normal glucose tolerance women with history of gestational diabetes**

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**Background and aims:** Women with a history of Gestational Diabetes (GDM) are known to be at risk to develop Type 2 Diabetes (T2DM), especially in the presence of obesity and of other conditions such as impaired fasting glucose or/and impaired glucose tolerance. However, the condition of former GDM represents by itself a risk factor for T2DM, even in the case of lean women with current normal glucose tolerance (NGT). Therefore, it is possible that some indexes of glucose metabolism could be found already altered in women with former GDM, although they are lean and with NGT condition at time of study.
**Materials and methods:** 24 women with former GDM, lean and with NGT condition were studied (age=31.3 ± 0.7 years, BMI=22.3 ± 0.3 kg/m$^2$, fasting glucose=81.8 ± 0.7 mg/dl, 2 h plasma glucose=90.2 ± 2.4 mg/dl; mean±SE); 23 women without former GDM, and without any risk factors for diabetes were also studied as control group. They were matched to former GDM women for all the above parameters (age=30.0 ± 1.1, BMI=22.0 ± 0.5, fasting glucose=82.0 ± 1.0, 2 h plasma glucose=90.6 ± 3.1, $P>0.33$ in the worst case). All the subjects underwent a standard 75 g oral glucose tolerance test (OGTT). Venous blood samples were collected at basal (before glucose ingestion) and for three hours afterwards, at 10, 20, 30, 60, 90, 120, 150 and 180 min. Then, we computed some indexes of insulin resistance/sensitivity, and of B-cell function: 1) HOMA-IR; 2) a model-based index of insulin sensitivity, OGIS, which assesses glucose clearance in dynamic conditions during an OGTT; 3) the insulinogenic index, IGI, calculated as the ratio of the difference between insulin concentration at 30 min and basal insulin to the corresponding difference in glucose; 4) the mean slope of the dose-response function, which represents the static relationship between insulin secretion and glucose concentration, calculated through a mathematical model of B-cell function; we call this index "glucose sensitivity"; 5) the derivative parameter, Pd, which represents the dynamic dependence of insulin secretion on the rate of change of glucose concentration, calculated with the same model as in 4); 6) the disposition index, DI, calculated as the product between OGIS and glucose sensitivity.
**Results:** With regard to indexes of insulin resistance/sensitivity, no significant difference ($P<0.05$) was found between former GDM and control group (HOMA-IR: 1.4 ± 0.1 vs. 1.7 ± 0.1 mmol/l · μU/ml, $P>0.12$; OGIS: 490 ± 9 vs. 511 ± 12 ml · min$^{-1}$ · m$^{-2}$, $P>0.21$). When considering the indexes of B-cell function, IGI was found not different (1.3 ± 0.4 vs. 0.9 ± 0.1 μU/ml · dl/mg, $P>0.48$), but a significant difference was found in glucose sensitivity (108 ± 10 vs. 167 ± 22 pmol · min$^{-1}$ · m$^{-2}$ · (mmol/l)$^{-1}$, $P<0.02$). Furthermore, also DI was found to be different (53 ± 5 vs. 86 ± 12 $10^3$ · ml · min$^{-2}$ · m$^{-4}$ · pmol · (mmol/l)$^{-1}$, $P<0.02$). No difference was found for Pd (773 ± 71 vs. 1005 ± 130 pmol · m$^{-2}$ · (mmol/l)$^{-1}$, $P>0.15$).
**Conclusion:** Indexes of B-cell function, calculated by mathematical modelling applied to OGTT data, were found to be lower in a group a women with former GDM compared to a group of control women, matched for age, BMI, fasting and 2 h plasma glucose. These results suggest that defects in B-cell function (in particular, the defect in glucose sensitivity) may appear early in the progression towards Type 2 Diabetes: in fact, these defects can be already present in former GDM women when the glycemic levels are still normal. Conversely, no difference was found between former GDM and control women in insulin sensitivity.

## 980

**A study on insulin sensitivity indexes in pregnant women with normal glucose tolerance and preeclampsia**

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**Background and aims:** Many studies in the last years have showed that insulin resistance is a condition related to hypertension and cardiovascular diseases. Pregnancy is physiologically a condition in which insulin resistance develops; furthemore several lines of evidence suggest that preeclampsia may be associated with greater degrees of insulin resistance than characteristic of normal pregnancy. Aim of our study was to evaluate insulin sensitivity in pregnant women with normal glucose tolerance and normotensive both in early and in late pregnancy. To achieve this aim, some indexes of insulin sensitivity derived from an oral glucose tolerance test or fasting glucose/insulin levels, were used to predict insulin sensitivity during pregnancy. Recently these indexes, as $IS_{HOMA}$, $IS_{QUICKI}$, OGIS have been validated vs clamp also in pregnancy. Furthemore we wont to verify the possible ability of these indexes in predicting the risk of subsequent preeclampsia development.
**Materials and methods:** 829 pregnant women mean age (31 ± 4 yrs) and mean BMI (22,5 ± 3) were subjected to OGTT with 75 gr of glucose between the 16–20 gestational weeks (gw) (early pregnancy) and 26–30 gw (late pregnancy). Plasma glucose, plasma insulinemia levels (fasting and after 1 h and 2 h) and development of preeclampsia were taken in consideration. As for sensitivity indices we calculated: $IS_{HOMA}$ = FPG*FPI/22.5; $IS_{QUICKI}$ = 1/(log FPI+log FPG); OGIS = $f(G_0, G_{60}, G_{120}, I_0, I_{60}, D_o)$ were G and I are glucose and insulin concentrations and $D_O$ was the oral glucose dose (g/m$^2$ body surface area); the expression of $f$ contains some parameters chosen to maximize the agreement with the clamp.
**Results:** Mean values of Fasting Plasma Glucose (FPG) were 64.7 ± 9.8 mg/dl and of Fasting Plasma Insulinemia (FPI) were 6.5 ± mU/ml in early pregnancy, FPG mean values were 61.4 ± 11.2 mg/dl and FPI mean levels were 8.1 ± 6.2 mU/ml in late pregnancy. $IS_{HOMA}$ was 1.33 ± 0.89 in early pregnancy and 1.46 ± 1.1 in late pregnancy; OGIS was 457 ± 69 ml/min$^{-1}$ m$^2$ and 444 ± 64 ml/min$^{-1}$ m$^2$ in early and late pregnancy respectively. $IS_{QUICKI}$ was 0.43 ± 0.27 in early pregnancy and 0.38 ± 0 in late pregnancy. We found a significantly correlation (p<0,005) between $IS_{HOMA}$ $IS_{QUICKI}$ and OGIS in early and late pregnancy These data confirm the reduction of insulin sensitivity during pregnancy.Preeclampsia developed in 6.4% of pregnant women. Preeclampsia was positively related to 75 centile of $IS_{HOMA}$ both in early and late pregnancy with a sensivity of 80% in both periods and a specificity of 96% in the early period and of 98% in the late period. Also $IS_{QUICKI}$ < 25° centile ($\chi^2$0.001) was related with preeclampsia development with a sensitivity of 81% and specificity of the 95% and 97% respectively.
**Conclusion:** Our data suggest that $IS_{HOMA}$ and $IS_{QUICKI}$ are simple tests that, already in the early phase of pregnancy, can put in evidence impaired insulin sensivity. Furthemore these indexes result precious in predicting the development of preeclampsia.

## 981

**Effect of parity on insulin resistance during pregnancy and on prevalence of gestational diabetes**

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**Background and aims:** To study the effect of parity on impairment of insulin sensitivity during pregnancy as well as on the appearance of gestational diabetes (GDM).
**Materials and methods:** We studied the relationship between parity and peripheral insulin sensitivity, evaluated by the index of Matsuda-De Fronzo ($ISI_{OGTT}$) or GDM prevalence in 1880 women, who performed a 100g-3 hr-oral glucose tolerance test (OGTT) between the 24$^{th}$ and 28$^{th}$ gestational week and in a subgroup of 75 women who carried out an OGTT in two consecutive pregnancies. A proxy for β-cell function (basal plasma C peptide/fasting plasma glucose; CP/FPG) was also obtained.
**Results:** Parity was associated with increased actual and pregestational BMI, weight increase during pregnancy, decreased $ISI_{OGTT}$, and increased CP/FPG only in those with parity >3. Glucose tolerance, estimated by 2 hr-AUC-glucose, was significantly impaired in those with parity >3. GDM was diagnosed in 124 women (6.59%), being its prevalence linearly related to parity (p=0.0034). The relationships between parity and $ISI_{OGTT}$ (inverse) or 2 hr-AUC-glucose (positive) were no longer significant after adjusting for age, pregestational BMI, and weight increase, while $ISI_{OGTT}$ resulted inversely related to pregestational BMI (p=0.0002) and weight increase (p=0.002) in GDM and in women with normal glucose tolerance (p=0.0001 for both). Two-hr-AUCG-glucose was directly related to age and pregestational BMI in the group with GDM (p<0.0001) and only to pregestational BMI in those with normal glucose tolerance (p=0.04), while CP/FPG was found significantly related to age, prepregnancy BMI and weight increase in the group of women with normal glucose tolerance, and to pregestational BMI and weight increase in those with GDM. $ISI_{OGTT}$ was reduced by about 40% (3.8 ± 2 mg/dl*min *vs.* 6 ± 3 mg/dl*min, p<0.0001) in women with GDM while CP/FPG was about 15% higher (0.15 ± 0.06nmol/mmol *vs.* 0.13 ± 0.06nmol/mmol; p=0.005). CP/FPG and $ISI_{OGTT}$ (both log-transformed) were, as expected, inversely related to each other (r=–0.51; p<0.0001 in the normotolerant group and r=–0.58; p<0.0001 in those with GDM) with a slope significantly flatter in the GDM group (–0.106 *vs.* –0.123; p<0.05), suggesting an impaired β-cell function in GDM. By multiple logistic model the risk of GDM, expressed as Odds Ratio, was increased by about 10% for every year of ageing and by about 7% for every BMI unit increase. In the longitudinal study glucose tolerance and $ISI_{OGTT}$ were significantly impaired while CP/FPG was significantly increased at the subsequent pregnancy, as compared with the index pregnancy. According to the multivariate time-series analysis the higher the increment in weight increase during the pregnancy and the longer the time interval between two consecutive pregnancies, the greater the decrease in $ISI_{OGTT}$ and increase in 2 hr-AUC glucose. A rise in BMI between pregnancies was related to reduction in $ISI_{OGTT}$, and, more marginally to decrease in glucose tolerance (p=0.07).
**Conclusion:** Parity is linked to insulin sensitivity deterioration and to CP/FPG increase or GDM appearance during the last trimester of pregnancy through progressive ageing and pregestational BMI increase. $ISI_{OGTT}$ decrease is related to a woman's weight gain, either before or during the pregnancy, needing the synergistic effect of a sufficiently long time interval between pregnancies.

## 982

**1,5-Anhydro-D-glucitol (1,5-AG) plasma level as a marker of hyperglycemic spikes in pregnant women with diabetes**

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**Background and aims:** Numerous studies confirmed that inadequate treatment of diabetic pregnant women results in increased mortality and perinatal morbidity of both mothers and neonates. It was revealed that oxidative stress due to postprandial hyperglycemia is the significant reason of such severe complications.
Therefore, methods monitoring carbohydrate metabolism in pregnant women, have to be particularly sensitive for detecting hyperglycemic episodes.
Concentration of 1,5-Anhydro-D-glucitol (1,5-AG) in human plasma seems to be the sensitive parameter for glucose excursions monitoring as 1,5-AG reabsorption is competitively inhibited by glucosuria, resulting in plasma 1,5-AG reduction.
Because studies concerning the usefulness of 1,5-AG plasma level estimation for monitoring of pregnant women with diabetes are not consistent, we focused on the changes in serum levels of this compound in diabetic pregnancy.
**Materials and methods:** The study group consisted of 55 pregnant women (mean age – 26.0 years, mean gestational age: 28.0 weeks) with gestational diabetes (GDM) (28 patients) or with pregestational diabetes (PGDM) (27 patients). In each patient 24-hour glucose serum profile (12 points, every 2 hours) and 1,5-AG plasma level (enzymatic method by Yabuuchi in own modification) were measured. Basing on 24-hours glucose profile, MBG (mean blood glucose) and M-value by Schlichtkrull were calculated. The mean maximal daily glycemia (MxG) was established as the mean of the maximal daily plasma glucose values of all patients.
**Results:** The significant correlation was found between 1,5-AG plasma level and maximal daily glycemia [r = (– 0.3)] and between 1,5-AG and M-value [r= (–0,36)]. The multivariate analysis showed, that the only parameter related to 24-hours glucose profile that determines 1,5-AG was maximal daily glycemia [beta = (–0,81), p = 0.01]. The M-value, the indicator of glucose profile fluctuations, significantly determines 1,5-AG but with lower statistical power [β = (–0.49)].
**Conclusion:** 1,5-AG could be helpful in detection of hyperglycemic excursions in pregnant women with diabetes.

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# Clinical audit and diabetes care

## 983

**Aspirin usage among Greek diabetic patients in terms of primary and secondary prevention strategy for coronary heart disease**

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**Background and aims:** Diabetes mellitus (DM) is regarded as a coronary heart disease (CHD) risk equivalent and low-dose aspirin therapy is recommended as a primary prevention strategy for these patients. Our aim was to determine the extent to which patients with type 2 diabetes, with or without CHD, comply with the recommended guidelines concerning aspirin therapy for primary and secondary prevention strategies.
**Material and methods:** A total number of 707 patients (371 men, 336 women) with a mean age of 61,49 ± 9,84 years were studied; 227 had type 2 diabetes and CHD (D/CHD+), 286 had type 2 diabetes without CHD (D/CHD-) and 194 were non-diabetic subjects with CHD (nD/CHD+). Diagnosis of CHD was based upon coronary angiography findings (≥50% coronary artery stenosis in at least one major artery), conducted during the period of March 2001–December 2003. Patients were examined about aspirin usage (enteric-coated aspirin in doses of 75–325 mg/day) as means of primary and secondary prevention strategy of CHD. Study according to the number of major coronary arteries with ≥50% stenosis was conducted as well. Statistical analysis was performed using one-way Analysis of Variables (AnOVa) in the SPSS 11.5 programme.
**Results:** Difference in the mean values of HbA1 c of the two diabetic groups was of no statistical significance. Aspirin usage in the D/CHD+ group (secondary prevention) was reaching up to 79,30% (180/227), whereas only 30,18% (86/285) were receiving aspirin in the D/CHD- group (primary prevention) (p<0,001). The usage of aspirin in the nD/CHD+ group (secondary prevention) was 80,91%, similar to the D/CHD+ group (D/CHD+ vs nD/CHD+ : 79,30% vs 80,91%, N/S). No statistically significant differences were observed in the D/CHD+ and the nD/CHD+ groups concerning aspirin usage (secondary prevention) according to the number of coronary arteries (C.Art) with evidenced obstruction (D/CHD+: 1 C.Art.: 92,08% usage of aspirin, 2 C.Art.:80,14%, 3 C.Art.:73,84%, N/S, nD/CHD+: 1 C.Art.: 88,57%, 2 C.Art.:84,37%, 3 C.Art.: 77,78%, N/S).
**Conclusion:** The proportion of Greek patients with type 2 diabetes receiving daily aspirin in terms of primary prevention strategy is extremely low regarding the recommended guidelines. On the contrary, usage of aspirin for secondary prevention appears to be in more satisfactory levels. Further intervention is needed in order to incorporate aspirin therapy for diabetic patients as standard practice for all primary care providers.

## 984

**Clinical practices among U.S. primary care practitioners when initiating or converting insulin therapy in patients with Type 2 diabetes mellitus**

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**Background and aims:** In the USA, the majority of patients with type 2 diabetes mellitus receive their care from primary care physicians (PCPs). In this retrospective chart review, we surveyed U.S. PCPs for their routine clinical practices when treating patients with type 2 diabetes either newly initiated to insulin (NEW) or undergoing a conversion of insulin therapy (CONV). To narrow the scope of this study, we evaluated PCP behaviors when initiating or converting patients to insulin lispro mix 25 / 75 [Humalog® Mix25™].
**Materials and methods:** One hundred-fifty (150) PCPs were recruited as first respondents from 2 mailings (N=300, N=500) to a national group of randomly selected PCPs with experience in prescribing insulin lispro mix 25/75. One hundred-fifteen (115) PCPs reviewed a maximum of 10 patient charts each, providing data for 996 patients (mean 8.7 patient charts/PCP). Of these 115 PCPs, 102 responded to a post-study physician questionnaire regarding their attitudes and beliefs in caring for their patients with type 2 diabetes. Information obtained from the patient charts and the post-study questionnaire was summarized as percentages or means ± standard deviation (SD).
**Results:** Demographic characteristics of the NEW and CONV patients were similar. Before initiating patients to insulin therapy, PCPs most often used 2 oral antihyperglycemic agents (OA) (46% of patients), but reduced this to 1 OA after starting insulin therapy (48% of patients). In contrast, for CONV patients, the number of OA PCPs used before converting insulin did not change at the time of conversion. The most common previous insulin regimen for CONV patients was twice-daily human insulin 30/70 (51%). Over time, PCPs routinely decreased sulfonylurea (SFU) use and increased thiazolidinedione (TZD) use whether initiating or converting insulin. When dosing insulin, PCPs did not compensate for the number of concommitant OA and used approximately double the insulin dose for twice-daily administration compared to once-daily administration. According to the post-study questionnaire, PCPs reported threshold values for fasting and post-prandial blood glucose and HbA1 c that would prompt them to initiate insulin therapy, and the average of these values closely approximated 2001 American Diabetes Association guidelines. PCPs ranked OA in the order they preferred to use them: metformin (MET), TZDs, SFUs, SFU/MET combination, meglitinides, and α-glucosidase inhibitors. The majority (72%) of PCPs responded they would use 3 OA before initiating insulin, but only 24% of patients received 3 OA before initiating insulin therapy, while 28% received 1 OA and 46% received 2 OA.
**Conclusions:** This retrospective chart review revealed a number of consistent behaviors when PCPs initiated or converted insulin therapy. PCPs routinely decreased the number of OAs used to 1 OA when initiating insulin therapy, whereas PCPs did not alter the number of OAs used when converting insulin therapy. Whether initiating or converting therapy, PCPs consistently decreased SFU use and increased use of TZDs. The post-study questionnaire revealed a discrepancy between PCP beliefs and actual clinical practice. Seventy-two percent of PCPs reported they would use 3 OA in combination before initiating insulin; however, this was not substantiated upon review of their actual clinical practice that showed PCPs tended to initiate insulin therapy before utilizing a third OA.

## 985

**Level of goal attainment according to the Third Joint Task Force treatment goals in a cohort of French patients with Type 2 diabetes**

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**Background and aims:** The Third Joint Task Force of European and other Societies on Cardiovascular Disease Prevention has defined treatment goals in patients with type 2 diabetes in order to reduce the risk of micro- and macro-vascular complications. This study aimed to determine the level of goal attainment in a cohort of French patients with type 2 diabetes and assess the factors associated with goal attainment.
**Materials and methods:** : In 2002, 135 French general practitioners and specialists were surveyed to collect demographic and clinical information on a random sample of their patients with type 2 diabetes whose age ≥ 30 years. The level of goal attainment was assessed according to the European guidelines from the Third Task Force ($HbA_{1C} \leq 6.1\%$, blood pressure (BP) < 130/80 mmHg, total cholesterol (TC) < 4.5 mmol/l and LDL-C < 2.5 mmol / l). Fasting and post-prandial blood glucose levels were not available and therefore not included. Factors associated with $HbA_{1c}$ control were investigated by multi-variable logistic regression.
**Results:** 3746 patients were included in the study. The average (SD) age was 62.4 years (11.6) and 53.9% were men. The average duration of type 2 diabetes was 8.4 years (8.0). Most were taking either oral hypoglycemic agents alone (76.1%) or in combination with insulin (12.4%). The level of goal attainment among patients with recent measure of HbA1 c, BP, TC and LDL-C is in table. Patients with the following characteristics were less likely to attain the recommended goal for $HbA_{1c}$: older age (Odds Ratio and 95% CI: 0.98, 0.97 – 0.99), longer duration of diabetes (0.97, 0.95 – 0.99), with neuropathy (0.55, 0.34 – 0.88) and dyslipidemia (0.63, 0.40 – 0.99), more frequent physician office visits (0.95, 0.92 – 0.98) and glucose monitoring (0.62, 0.50 – 0.78), and viewed as non-compliant to hypoglycemic medications by his/her physician (0.17, 0.12 – 0.23).

| | $HbA_{1c}$ | BP | TC | LDL-C |
|---|---|---|---|---|
| Number (%) of patients with measurement | 3433 (91.6) | 2048 (54.7) | 1697 (45.3) | 1951 (52.1) |
| Average (SD) | 7.6 (1.6) % | 80.5 (11.3)/ 142(15.5) mmHg | 5.1 (1.2) mmol/L | 3.2 (1.1) mmol/L |
| % achieving goal | 13.1 | 7.8 | 26.8 | 23.3 |

**Conclusion:** In this cohort of French patients with type 2 diabetes, the level of goal attainment was low according to the European guidelines. Innovative medicine and management strategies are needed to prevent cardiovascular disease in these patients.

## 986

**Metabolic status and control in patients with diabetes: results from the Aarhus County Diabetes Database, Denmark**

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**Background and aims:** We have established a database that allows for routine monitoring of epidemiological and clinical indicators in an administratively well-defined population of patients with diabetes. We here report results related to metabolic status and control.
**Materials and method:** Data from The National Health Service Registry, The Regional laboratory database and The Danish National Hospital Registry have been used for the identification and characterization of the diabetic patients in the population of Aarhus County, Denmark (total population size about 650,000 inhabitants, corresponding with 12% of the Danish population). The data analyzed refer to the period 1 January 2000 through 31 December 2002.
**Results:** A total of 19,105 diabetic patients have been enrolled in the database. A total of 108,072 measurements of HbA1 c have been registered, representing 15,517 of the patients. Over the three years period 2000 through 2002, mean values of HbA1 c for patients measured quarterly distributed as follows. HbA1c < 6.5 %: 21.7%; 6.5% ≤ HbA1c < 8.0 %: 38.0%; 8.0% ≤ HbA1c<9.5%: 26.0%; 9.5%≤HbA1 c<11.0%: 10.1%; 11.0%≤HbA1 c: 4.1%, without changes during the period. In the patient population prevalent as of December 31 2001 (n=15,606 patients), 67.8% had had at least one measurement of HbA1 c in both 2001 and 2002; 13.8% had had no measurement of HbA1c in neither 2001 nor 2002 whereas 18.4% had had HbA1c measured in 2001 or in 2002.
**Conclusions:** The metabolic status and level of control in this population-based large sample of Danish patients with diabetes is poor. Only about 60% of the patients have satisfactory levels of HbA1 c and about 40% must be considered at high risk of long-term complications due to high levels of HbA1 c. A substantially fraction of the patients (almost 14%) have infrequent measurements of HbA1 c. Using a database system like the one presented here will assist in the future monitoring of metabolic status and control in the patient population. In-depth studies of samples of the patients may reveal potential barriers for improved diabetes care.
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## 987

**Effectiveness of a structured program on the quality of outpatient diabetes care: experiences from Hidalgo, Mexico**

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**Background and aims:** In spite of the evidence from clinical trials about the effectiveness of metabolic control in reducing clinical outcomes, direct costs and improving quality of life, deficiencies in diabetes care persist worldwide. Leading factors include insufficient coverage/barriers to access to medical care, lack of diabetes educators, organizational issues and limited economic resources. The objectives of our work were to investigate the effectiveness of an structured diabetes program on the quality of care provided by multidisciplinary groups in Mexico, and its effect on glycemic control.
**Materials and methods:** Starting in 2001, a diabetes program was implemented in Hidalgo, a central state in Mexico, with a largely rural, noninsured population, in which the largest part of medical care is provided by general practitioners at health centers. A diabetes registry was established, in which data from 11,700 patients have been collected, and thirty clinics were established at each of the 13 regions of the state, resulting in unprecedented coverage for diabetes. Multidisciplinary groups were assembled at each clinic, with a general practitioner, a nurse, and a diabetes educator at each clinic. Clinical guidelines were customized and discussed with providers, and quarterly workshops are scheduled to review process, advances and continuing development. Quality of care is measured by means of a modified version of the Diabetes Quality Improvemet (DQIP) standards.
**Results:** 1,700 patients have been enrolled at the first 13 diabetes clinics; quality markers from 841 patients who have completed five visits are presented: Significant reductions in fasting blood glucose (p<000.1), HbA1c (p 00.1), and diastolic blood pressure (p 0.018) have been documented, and the rate of foot lesions has decreased fivefold: from 3.6 percent at baseline to 0.08 percent at the fifth visit.
**Conclusion:** The results of our study show that improvements in the quality of outpatient diabetes care can be accomplished at the primary level, in spite of limited resources: HbA1 c, self-monitoring of blood glucose and lipoprotein measurements are not provided in Mexico. Consequently, patients have to pay these examinations from their pockets. Nevertheless, continuing increments in these variables have ocurred at the diabetes program in Hidalgo, while performance of clinical measurements is high, concurrent with improvements in glycemic control.

Percentage of Quality Markers by Visit

| Variable or intervention | Visit 1 | Visit 2 | Visit 3 | Visit 4 | Visit 5 | Overall |
|---|---|---|---|---|---|---|
| BMI | 65.2% | 95.1% | 94.9% | 93.9% | 92.8% | 88.77% |
| Blood pressure | 69.4% | 94.5% | 93.1% | 95.1% | 94.5% | 94.26% |
| Fasting blood glucose | 90.07% | 80.7% | 77.56% | 72.6% | 69.03% | 96.49% |
| HbA1c | 5.56% | 5.84% | 3.81% | 2.38% | 2.49% | 26.15% |
| Total cholesterol | 17.2% | 16.9% | 10.7% | 7.2% | 7.1% | 54.9% |
| Triglycerides | 6.8% | 10.02% | 6.4% | 3.8% | 3.8% | 34.9% |
| Foot examination | 84.7% | 63.98% | 87.4% | 90.1% | 91.9% | 91.9% |

## 988

**Continuous population-based measurement of quality of diabetes care using 79000 HbA1c values in the state of Thuringia/Germany – results in 2003**

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**Introduction/Hypothesis:** There remains insufficient knowledge about the quality of diabetes therapy regarding structure, process and outcome given by primary care in Thuringia, and elsewhere in Germany. Since 1997, the ongoing project assesses a means of determining quality of diabetes therapy in primary care by analysis of HbA1 c values within a broad territory.
**Method:** Creation of a map which displays the adjusted medium HbA1 c of the districts of Thuringia: Collection and analysis of all HbA1 c tests (January 1st – March 31st 2003) which were done on the primary care level in Thuringia. HbA1 c tests were collected in the medical laboratories. Each HbA1 c test was identified by the postal code of the Thuringian General Practitioner (GP) who ordered the test and adjusted to the DCCT reference range (mean normal HbA1 c of healthy subjects: 5,05%). Personal data of physician and patient were not analysed. Thuringia (population of 2.392.040) consists of 23 urban and rural districts, this format was also used in the present study. All laboratories participated in an inter-laboratory test for HbA1 c. Completeness of data was evaluated by a second data source (KV Thüringen, Clearing House for Thuringian physicians (CH)).
**Results:** 23 medical laboratories and diabetes clinics provided 93.961 HbA1 c-tests (2002: 74.936). 79.086 HbA1 c-tests (2002: 59.702) belonged to Thuringian patients (total number of HbA1 c-tests in Thuringia due to CH: 104.168). The mean adjusted HbA1 c of entire Thuringia was 6,59% (2002: 6,75 %). 31,1% (2002: 36,4%) of all HbA1 c-tests were above 7,0% (near-normoglycaemia). 2,4% (2002: 3,0%) of all HbA1 c-tests were above 10,0% (acute complications from hyperglycaemia). The mean adjusted HbA1 c of the rural and urban districts of Thuringia varied between 6,1% (2002: 6,3%) and 6,9% (2002: 7,1%). Compared to 2002, the mean adjusted HbA1 c was reduced in 19 of 23 districts. All laboratories passed the inter-laboratory test for HbA1 c.
**Conclusion:** The completeness of data was increased to 75%. In most Thuringian districts there was a reduction of the adjusted mean HbA1c and the percentage of HbA1 c-tests above 7,0% and 10,0%. This finding could be indicative for an improvement of the outcome quality of diabetes care in Thuringia. The results of the inter-laboratory test are indicative for a sufficient quality of HbA1 c analyses. However, the proposed method is still experimental and has to be evaluated by population based data. More data about other aspects of diabetes care, such as diabetes related medications and blood pressure control, should improve the method.

## 989

**EAGLE - Economic Analyses of Glycemic control and Longterm Effects: A computer simulation model for diabetes mellitus Type 1 and Type 2**

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**Background and aims:** Since type 1 and type 2 diabetes are chronic diseases, the development of long-term complications and their economic consequence are difficult to assess through short-term studies. Therefore, a model was developed to simulate the long-term effects of different diabetes therapies on diabetes-related complications and costs.
**Materials and methods:** The model development included the following steps: development of the model structure independent of data availability; systematic review of published data in type 1 and type 2 diabetes; the model was constructed as a stand-alone computer simulation program providing a micro-simulation of a virtual patient cohort over n years in 1-year cycles with a maximum of 1000 iterations and subsequent health economic (HE) calculations. HE calculations are constructed from the simulation results (diabetes-related complication rate).
**Results:** 1. Epidemiology: Trial results from DCCT, UKPDS, and WESDR served as the basis for the calculation formulae in the model. Complications include hypoglycemia, retinopathy, neuropathy, end stage renal disease, neuropathy, amputation, cardiovascular complications, stroke, and death, and are calculated over time as cumulative incidence. Risk equations for the probability of complications were based on regression analysis that contain linear, exponential, as well as quadratic regression formulae. The probability to develop a complication is stored in the risk equations, which include several demographic (eg, age, gender, duration of diabetes since diagnosis, type of diabetes) and physiological parameters (eg, A1 c, systolic blood pressure, triglycerides, HDL, LDL, albumin excretion rate). The course of HbA1 c over time is simulated in relation to the treatment regimen. Up to 8 patient cohorts of 100,000 patients with different baseline values and treatment regimens can be simulated at the same time.
2. Health Economics: Costs for diabetes treatments and complications can be assigned and stored for different individual countries. For all simulated patient cohorts, cost of complications, cost of illness, cost effectiveness, cost consequence, and cost utility analysis can be calculated.
The data output of each simulation includes incidence per year, cumulative incidence after n years, all demographic and physiological parameters, and results of the health economic analyses. All data are presented for each simulated patient cohort separately.
**Conclusion:** The EAGLE model is a user-friendly, flexible, and robust tool for the analysis of the long-term effects of diabetes treatments and its related costs in type 1 and type 2 diabetes.

## 990

**Systematic reviews do not allow appraisal of complex interventions of diabetes or hypertension self-management**

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**Background and aims:** Self-management programmes are complex interventions. A phased approach defining sequential stages of a continuum of increasing evidence has been proposed as a framework for the design and evaluation of such complex interventions (BMJ 2000;321:694–696). The aim of the present study was to explore if the continuum of increasing evidence for three diabetes and hypertension programmes could be extracted from systematic reviews.
**Materials and methods:** Three complex interventions of diabetes and hypertension self-management (Diabetologia 2002; 45 : 1723 – 1733) were used for analysis. As the author was instrumental in generating evidence for these programmes over a period of 20 years she had direct access to all publications (n=56). Relevant systematic reviews (Cochrane and Medline Databases, 1997 to 2002) were analysed for reasons including or excluding articles that were part of the phased evaluation of the three programmes.
**Results:** 391 reviews on diabetes and 95 on hypertension and all meta-analysis (114 diabetes, 151 hypertension) in PubMed and all Cochrane reviews were screened. References of articles and diabetes journals were reviewed. Finally, 9 reviews were eligible. Single, but different publications of the three complex interventions were included in the reviews. None of the three programmes was identifiable as a complex intervention with a continuum of increasing evidence. Reasons: Exclusion of complex interventions if single components could not be isolated; varying classification of identical programmes within and between reviews; definition of common outcome measures for different programmes disregarding the complexity of effectiveness measures of the original studies, e. g. HbA1 c as an isolated outcome variable without considering treatment goals, intended changes in medication or weight or inseparable effects on hypoglycaemia. By only screening abstracts publications may not be recognized as replication or implementation trials.
**Conclusion:** Methodology of systematic reviews has to be adjusted to allow synthesis of evidence on complex interventions such as diabetes and hypertension self-management programmes.

# PS 95

# Observational studies in Type 2 diabetes

## 991

**Lifetime cost-effectiveness of rosiglitazone-metformin combination in obese patients with Type 2 diabetes in Germany**

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**Background and aims:** Guidelines in Germany recommend use of Rosiglitazone in combination with Metformin for treatment of Type 2 diabetes patients when Metformin monotherapy is no longer effective in maintaining glycaemic control. We assess the cost-effectiveness of this strategy compared to conventional care of Metformin in combination with Glibenclamide (SU)

**Materials and methods:** DiDACT, an established long-term economic model of Type 2 diabetes, was adapted for clinical practice and health care financing rules in Germany. The model was calibrated using CODE-2 study data and national statistics. The perspective is that of the sickness funds, and includes all hospital care, physician consultations, medications, rehabilitation, physiotherapy, foot care and sick leave. The model was used to simulate treatment histories for a mixed incident cohort of 1,000 newly diagnosed obese patients (mean BMI=34 kg/m$^2$). Following failure of glycaemic control with Metformin monotherapy, combination therapy adding Rosiglitazone (8 mg daily) was compared to adding titrated SU. Insulin monotherapy was initiated following failure of oral combinations. The threshold for switching therapies was 7% HbA1 c as recommended by German guidelines. In line with national guidelines, costs were discounted at a rate of 5% pa and health outcomes were not discounted.

**Results:** The model predicts that adding Rosiglitazone to Metformin produces better glycaemic control in most patients, and extends viability of oral anti-diabetic therapy by 3.5 years before requiring insulin. The improvement in glycaemic control results in reductions in morbidity due to reduced risk of developing or progressing to later stages of complications. This is projected to generate 312 additional Quality Adjusted Life Years (QALYs) in the cohort over their lifetimes. The additional QALYs comprise 116 (37%) from better survival and 196 (63%) from delaying insulin and reduced or delayed complications. Estimated benefits are conservative as some patients progress too rapidly to insulin to be eligible for combination therapy. Cost increases are partly offset by savings from delaying insulin therapy. Discounted (undiscounted) incremental cost-effectiveness ratios were €17,628 (€21,463) per QALY gained modelled to end of life.

**Conclusion:** Estimated incremental cost-effectiveness ratios fall below US & UK „willingness to pay" thresholds, indicating that Rosiglitazone in combination with Metformin to improve glycaemic control and delay use of insulin and complications in obese patients is cost-effective in Germany when compared with conventional care of Metformin in combination with SU. The improvements lead to gains in both quantity and quality of life.

*Supported by: GlaxoSmithKline*

## 992

**The effect of pioglitazone (PIO) as monotherapy and in combination with other oral diabetic medication (OAM) on glycemic control and insulin resistance in patients with Type 2 diabetes**

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**Background and aims:** The objective of the present study was to evaluate the effect of PIO monotherapy (M) and combination (C) with other OAM on parameters of glycemic and lipid control and insulin resistance in patients (pts) with type 2 diabetes mellitus (T2DM).

**Materials and methods:** The study design was open, prospective, multicenter and observational. Patients (n=85) enrolled in the study were assigned to 2 groups. The M group consisted of 30 drug-naïve pts not adequately controlled on diet alone; 55 pts from the C group had poor glycemic control despite treatment with either glibenclamide (GLIB) or metformin (MET). The mean age of pts was 56.7 ± 9.2 years; mean duration of diabetes was 3.7 ± 3.5 years; and mean BMI was 31.4 ± 4.2 kg/m$^2$ (all data presented as M±SD). PIO was administered to all pts at a dose of 30 mg once daily and treatment lasted for 3 months in both groups. Effect of PIO therapy on insulin resistance parameters and β-cell functional activity (BFA) was evaluated using the Homeostasis Model Assessment (HOMA). The model was used for determination of Insulin Resistance Index (II) and BFA from the following formulas:
II = (IRI [μU/ml] × FPG [mmol/l]) ÷ (22.5) and BFA = (IRI [μU/ml] × 20) ÷ (FPG [mmol/l] - 3.5)
where IRI is immunoreactive insulin. Comparison in HbA1 c, FPG and HOMA-BFA were with baseline mean value.

**Results:** PIO therapy resulted in improvement of glycemic control parameters in both treatment groups. HbA1 c level decreased from 8.6 ± 1.4 at baseline to 7.3 ± 1.2% ($p<0.001$) in the M group and from 8.4 ± 1.2 to 7.6 ± 1.1% ($p<0.001$) in the C group. Fasting plasma glucose (FPG) decreased from 10.2 ± 2.8 at baseline to 8.6 ± 2.2 mmol/l ($p<0.001$) in the M group and from 9.9 ± 2.7 to 8.2 ± 2.0 mmol/l ($p<0.001$) in the C group. Subgroup analysis of the C group showed that both subgroups PIO+GLIB and PIO+MET) had significant decrease in HbA1 c and FPG but there was no difference between the subgroups at the end of study. After three months of PIO therapy, 50% of group M pts and 33.3% of group C pts reached the ADA target of good glycemic control (HbA1 c<7%). Inadequate glycemic control (HbA1 c>9%) remained in only 7.7% of pts receiving M and in 15.7% of pts receiving C. There was significant increase in HDL-C in the C group from 1.2 ± 0.2 to 1.4 ± 0.5 mmol/l ($p<0.001$); and in the M group from 1.2 ± 0.3 to 1.5 ± 0.7 mmol/l ($p=0.020$). In the M group, II decreased from 10.6 ± 6.4 to 7.4 ± 3.8 ($p<0.001$). In addition, BFA increased by 9.7 ± 60.4 and IRI decreased by 4.1 ± 12.2 μU/ml, although the changes were not statistically significant. In the C group, II decreased from 9.3 ± 5.9 to 5.6 ± 2.9 ($p<0.001$) and IRI from 20.8 ± 11.4 to 15.3+6.4 μU/ml ($p<0.001$). BFA increased by 3.0 ± 44.6, but this increase was not statistically significant. There was no difference in insulin resistance parameters between the subgroups PIO+GLIB and PIO+MET. A slight increase in body weight (from 85.7 ± 12.7 to 86.3 ± 12.7 kg; $p=0.002$) was observed in the subgroup PIO+GLIB by the end of study; in the same subgroup 2 (2.4%) pts had 4 episodes of mild hypoglycemia.

**Conclusions:** In pts with T2DM, the use of PIO as monotherapy as well as in combination with other oral hypoglycemic agents results in significant improvement of glycemic control and lipid profile. This improvement is associated with increased insulin sensitivity as assessed by HOMA.

## 993

**Impact of changes in therapy switching threshold on clinical and cost-effectiveness outcomes of treatments for obese patients with Type 2 diabetes in Germany**

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**Background and aims:** The therapy switching threshold is the blood glucose level at which a treatment is considered to be failing to maintain glycaemic control, necessitating a switch to an alternative treatment. We assess the impact of a change in therapy switching threshold on lifetime clinical and cost-effectiveness of conventional and proposed care for obese patients with Type 2 Diabetes in Germany.

**Methods:** DiDACT is an established long term model of Type 2 diabetes in Germany, which takes a sickness funds perspective and includes all hospital care, physician consultations, medications, rehabilitation, physiotherapy, foot care and sick leave. DiDACT was used to simulate treatment histories, and associated clinical and economic outcomes, for a mixed incident cohort of 1000 newly diagnosed obese patients (mean BMI=34). German guidelines recommend a switching threshold of $HbA_{1c}$ < 7.0%. We assess an increase to 7.5%, because in some patients a lower $HbA_{1c}$ threshold cannot be achieved in clinical practice. Following failure of glycaemic control with Metformin alone, proposed combination therapy adding Rosiglitazone (8 mg/d) was compared to conventional care of adding titrated Glibenclamide (SU). Triple combination therapy with insulin followed failure of Metformin + SU. Insulin monotherapy followed failure of Metformin + Rosiglitazone. In line with national guidelines, costs were discounted at 5 % pa.

**Results:** A higher therapy switching threshold simultaneously results in a deterioration in lifetime glycaemic control and an extension of the viability of oral anti-diabetic therapy. The deterioration in glycaemic control increases the risk of developing complications and ultimately increases morbidity and mortality. This negative effect is smaller in the Metformin + Rosiglitazone cohort, since Rosiglitazone produces better glycaemic con-

trol then SU. Conversely, the extended viability of oral therapy before requiring insulin leads to improvements in quality of life. This beneficial effect is greater in the Metformin + Rosiglitazone cohort, since the superior glycaemic control with Rosiglitazone further delays the need for insulin. When Metformin monotherapy fails we observe that addition of Rosiglitazone improves health outcomes better than addition of SU. Cost increases are partly offset by savings from delaying insulin therapy. Discounted incremental cost-effectiveness ratios (ICERs) fall below US & UK "willingness to pay" thresholds.
**Conclusion:** When choosing the most appropriate switching threshold there is a trade-off to consider between glycaemic control and quality of life. However, estimated ICERs for proposed care adding Rosiglitazone to Metformin compared to conventional care adding titrated SU are robust to changes in switching threshold.

Lifetime health outcomes and costs

| Treatment Strategy | Switching Threshold ($HbA_{1c}$) | Insulin Initiation (Years) | Discounted Costs (euro M)* | Life Years | QALYs | ICER per Life Year | ICER per QALY |
|---|---|---|---|---|---|---|---|
| Metformin | 7.0% | 7.5 | 50.4 | 16,425 | 9,672 | - | - |
| + SU | 7.5% | 13.5 | 54.9 | 16,240 | 9,938 | - | - |
| Metformin + | 7.0% | 11 | 55.6 | 16,614 | 9,967 | - | - |
| Rosiglitazone | 7.5% | 17.5 | 59.9 | 16,510 | 10,082 | - | - |
| Comparative Incremental | 7.0% | 3.5 | 5.2 | 188 | 295 | 27,516 euro | 17,523 euro |
| Analysis | 7.5% | 4 | 5.0 | 270 | 143 | 18,345 euro | 34,471 euro |

(* figures rounded)

*Financial support was provided by GlaxoSmithKline*

## 994

**Rosiglitazone plus sulphonylurea is effective and safe in daily practice**
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**Background and aims:** The aim of this study was to investigate the efficacy and safety of rosiglitazone (RSG) in combination with sulphonylurea (SU) in daily practice in Germany.
**Materials and methods:** Data from 6,406 patients, who were treated with RSG plus SU in two observational studies (AOCS = AVA-177: n = 5,499 and AVA-295: n = 907) were pooled and analysed. The patients were treated over a mean time of 6 months, which represents 3,213 patient years (PY) in total.
**Results:** Before the observational period, the efficacy evaluable patients (AVA-177: n = 5,033, AVA-295: n = 854, total: n = 5,887) represented a typical, insufficiently controlled diabetic population in Germany: age 64.0 years, diabetes duration 5.3 years, male:female ratio 1:1, BMI 27.7 kg/m$^2$, $HbA_{1c}$ 8.2%, fasting blood glucose (FBG) 10 mmol/l (each median). 64% were treated with glibenclamide (median dose: 7 mg/day) and 33% with glimepiride (median dose 3 mg/day). At the start of the observational period, RSG 4 mg/day was added to SU in > 97% of the patients. During the course of the study, RSG was uptitrated to 8 mg/day in 23% of the patients. Following the addition of RSG, $HbA_{1c}$ (-1.3 %) and FBG (-2.94 mmol/l) were both significantly reduced at the end of the 6 months observational period. The proportion of patients with $HbA_{1c} \leq 6.5\%$ (IDF goal) and $\leq$ 7.0% (ADA goal) increased from 5% and 13% at the start to 37% and 59% at the end of the observational period. Mean blood pressure decreased from 144/84 to 138/82 mmHg. Mean body weight was reduced by 0.7 kg from 81.4 kg to 80.7 kg. Non serious adverse events were reported for 2.3% of the total observed patients, serious adverse events for 1.0% of the patients.
**Conclusion:** These data show that therapy with rosiglitazone plus sulphonylurea is effective, safe and well tolerated in daily practice. A majority (59%) of the patients reached a $HbA_{1c}$ goal $\leq$ 7.0% after an observational period of 6 months.
*Supported by: GlaxoSmithKline GmbH & Co.KG*

## 995

**Rosiglitazone plus metformin is effective and safe in daily practice**
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**Background and aims:** The aim of this study was to investigate the efficacy and safety of rosiglitazone (RSG) in combination with metformin (MET) in daily practice in Germany.
**Materials and methods:** Data of 11,014 patients, who were treated with RSG plus MET in two observational studies (AOCS = AVA-177: 7,705 patients and AVA-295: 3,309 patients) were pooled and analysed. The patients were treated over a mean time of 6 months, which represents 5,542 patient years (PY) in total.
**Results:** Before the observational period, the efficacy evaluable patients (AVA-177: n = 7,160, AVA-295: n = 3,161, total: n = 10,321) were treated with daily MET-dosages of 500–2550 mg. They represented a typical, insufficiently controlled diabetic population in Germany: age 60.0 years, diabetes duration 3.7 years, male:female ratio 1:1, BMI 29.3 kg/m$^2$, $HbA_{1c}$ 8.1%, fasting blood glucose (FBG) 9.5 mmol/l (each median). At the start of the observational period, RSG 4 mg/day was added to MET in > 97% of the patients. During the course of the study, RSG was uptitrated to 8 mg/day in 26% of the patients. Following the addition of RSG, $HbA_{1c}$ (-1.3 %) and FBG (- 2.61 mmol/l) were both significantly reduced at the end of the 6 months observational period. The proportion of patients with $HbA_{1c} \leq$ 6.5% (IDF goal) and $\leq$ 7.0% (ADA goal) increased from 5% and 14% at the start to 39% and 64% at the end of the observational period. Mean blood pressure decreased from 144/85 to 137/82 mmHg. Mean body weight was reduced by 1.7 kg from 87.4 kg to 85.7 kg. Non serious adverse events were reported for 1.3% of the total observed patients, serious adverse events for 0.4% of the patients.
**Conclusion:** These data show that therapy with rosiglitazone plus metformin is effective, safe and well tolerated in daily practice. A majority (64%) of the patients reached a $HbA_{1c}$ goal of $\leq$ 7.0% after an observational period of 6 months.

## 996

**IRIS III study: effect of pioglitazone on parameters of insulin resistance and the metabolic syndrome**
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**Background and aims:** Pioglitazone is a PPARγ agonist which has shown to improve insulin-resistance and different features of the metabolic syndrome. This observational trial investigated the effect of a 5 months pioglitazone treatment on several clinical scores for the judgement of the metabolic syndrom.
**Materials and methods:** One-thousand-seventy-six patients with type 2 diabetes (562 male, 514 female; age 63.0 ± 10.3 years; duration of diabetes 6.7 ± 1.4 years; HbA1 c 7.8 ± 1.4 %; BMI 30.3 ± 5.4; W/H ratio 0.91 ± 0.12; mean ± SD) previously treated with sulfonylurea and/or metformin received 30 mg pioglitzone in combination with sulfonylurea or metformin. After 10 and after 20 weeks of treatment, BMI, WH-ratio, blood pressure, fasting glucose, and plasma lipids were assessed and the IRIS II, the WHO, and the ATP III score were calculated. HbA1 c was measured at baseline and after 20 weeks in a central laboratory.
**Results:** According to all scores used in our trial, a significant reduction in insulin-resistance could be observed during pioglitazone treatment. The percentage of patients classified as insulin-resistant according to the different scores are given in table 1. The IRIS II Score declined from 69 ± 18 (35–102) at baseline to 62 ± 21 (22–102) after 10 weeks, and to 59 ± 21 (18–101) at 20 weeks of treatment (95% confidence interval; $p<0.0001$, respectively). While patients with an IRIS II Score less than 40 showed no improvement according to the insulin resistance score, patients with an IRIS II Score ≥ 40 showed a mean decline of 11.3 ± 17.8 (0–35; $p<0.0001$) during the observational period. Patients with insulin-resistance according to the IRIS II score presented a significantly stronger improvement in HbA1 c compared to those without insulin resistance (-0.94 ± 1.3 vs. -0.6 ± 1.1; $p<0.0001$).
**Conclusion:** While the ATP III and the WHO score enable only a nominal classification of insulin-resistance, the IRIS II score allows longitudinal estimation in the course of insulin resistance. The IRIS II score proved to be

a useful clinical score for identification and monitoring of those patients which obtain highest benefit from a insulin sensitising therapy.

Table 1: Percentage of patients with insulin resistance (*p < 0.0001, compared to baseline)

| | Baseline | 10 ± 2 weeks | 20 ± 2 weeks |
|---|---|---|---|
| IRIS II | 53 | 39 * | 34 * |
| ATP III | 87 | 81 * | 76 * |
| WHO | 53 | 49 * | 47 * |

## 997

**The relation between the phenotypes and the efficacy of drug(s) in Type 2 diabetic patients: CoDiC-based analyses on the clinical data obtained at multiple institutions**

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**Background and aims:** Type 2 diabetes mellitus is heterogeneous disorder. Recently, various types of oral hypoglycemic agent (OHA) and insulin preparation (insulin) have been developed. Because the efficacy of these drugs is related to the pathophysiology, the selection of proper drug(s) among various types is important. To elucidate the relation between the phenotypes of patients and the efficacy of OHA and insulin, we analyzed the large scale of clinical data input in CoDiC, an electronic system for diabetes data collection and diabetes management, at multiple institutions.
**Materials and methods:** Type 2 diabetic patients have been registered in CoDiC at 34 institutions specialized in diabetes in Japan. Study I and II were carried out in the patients medicated with OHA and/or insulin. In Study I, we analyzed the actual using of agents and clinical data including HbA1 c and plasma CPR at random in 15,553 patients registered from January to July, 2003. In Study II, we analyzed changes in the efficacy of drugs during 30 months in 1,565 patients who had their first consultation in 2000. The analyses were carried out using Microsoft Excel and SPSS. On the participation, the purpose and risk were explained by board. The protocol was approved by the Japan Clinical Diabetes Data Management Study Group ethics committee.
**Results:** In Study I: mean HbA1 c was 7.12% in patients medicated with one species of OHA(n=5599), 7.49 in patients with more than 2 species of OHA(OHAs) (5030), 7.82 in patients with insulin and one species of OHA(insulin+OHA) (1192), 7.92 in patients with insulin and more than 2 species of OHA(insulin+OHAs) (568), 7.62 in patients with conventional insulin treatment (CT) (1591) and 7.87 in patients with intensive conventional insulin treatment (ICT) (1412). Percentage of patient numbers with poor glycemic control (HbA1 c>8.0%) was highest in patients with insulin+OHA(s) (34.4%). Mean BMI was relatively low in patients with insulin (CT: 23.4 kg/m$^2$, ICT: 23.9). In patients with insulin+OHA(s), mean BMI was highest and also its standard deviation (SD) was largest (insulin+OHA: 24.7 ± 3.9, insulin+OHAs: 25.4 ± 4.4). CPR was relatively low with insulin (CT: 2.1ng/ml, ICT: 1.9) and relatively high in patients with OHA(s) (OHA: 3.1, OHAs: 3.1). In patients with insulin+OHA(s), CPR was relatively high and its SD was largest (insulin+OHA: 2.7 ± 7.2, insulin+OHAs: 3.3 ± 7.9). In Study II, glycemic control has significantly improved in patients with OHA(s) or insulin (CT and ICT), however, not improved in patients with insulin and OHA(s). Percentage of patient numbers with poor glycemic control was highest in patients with insulin+OHA(s) (35.3%).
**Conclusion:** Because we analyzed clinical data in large scale of patient numbers and obtained at multiple institutions, the results seem to show actual status of pharmacological treatment in type 2 diabetic patients. The most interesting result is that it is difficult to improve glycemic control in patients treated with insulin and OHA(s). Because the adiposity and plasma CPR levels markedly varied in this patient group, the difficulty is caused by the marked heterogeneity of pathophysiology. We should take account of the pathophysiology for the selection of proper drug among various types.
*Supported by: JDDM*

# PS 96
# Gestational diabetes

## 998

**The prevalence of the metabolic syndrome in a Danish population of women with previous GDM is 3-fold higher than in the general population**

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**Background and aims:** The prevalence of overweight and diabetes are increasing worldwide, also among Danish women with a history of gestational diabetes (GDM). Glucose intolerance and obesity in combination with insulin resistance, hypertension and hyperlipidaemia comprise the metabolic syndrome and predicts increased cardiovascular morbidity and mortality. We aimed to estimate the prevalence of the metabolic syndrome according to three different criteria (WHO 1999, ATP III 2001 and EGIR 2002) in a Danish population of women with previous diet-treated GDM.
**Materials and methods:** Between 2000 and 2002, we examined 481 women with diet-treated GDM diagnosed during 1978–1996 for components of the metabolic syndrome – including OGTT, fasting serum insulin, weight/height, waist circumference, blood pressure, plasma triglyceride, HDL-cholesterol and albumin/creatinine ratio. A sample of 1000 age-matched women from a neighbor county who participated in a population-based study (Inter99) in the years 1999–2001 was examined as controls.
**Results:** The median age and BMI were 42.9/45.0 years and 27.9/24.6 kg/m$^2$ (both p<0.005) in the GDM/control group. The prevalence of the metabolic syndrome was three times higher in the prior GDM group compared to control group, independent of the criteria used (e.g. WHO: 38.4% vs. 13.4%, p<0.0005). Even after adjusting for BMI and age the risk for having the metabolic syndrome in the GDM group was increased (OR 3.4, 95% CI 2.5–4.8). In normal-weight women the prevalence of the metabolic syndrome was 4 times increased in the GDM group vs. the control group (11.4% vs. 2.6%). In glucose tolerant women the prevalence of the metabolic syndrome was doubled in the prior GDM group compared to control group.
**Conclusion:** The prevalence of the metabolic was significantly higher in women with prior diet-treated GDM compared to an age-matched control group, even in normal-weight and glucose tolerant subjects.

## 999

**Changes in glucose tolerance in women with previous gestational diabetes within one year after delivery: The Viennese Post-Gestational Diabetes Project**

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**Background and aims:** Women with a history of gestational diabetes (pGDM) are at high risk to develop type 2 diabetes (DM2). Therefore we aimed to i) assess the natural course of changes in glucose metabolism, ii) describe changes in beta cell function and insulin sensitivity and iii) identify predictive factors for the progression to an impaired glucose metabolism in a cohort of healthy European women with pGDM within one year following delivery.
**Materials and methods:** 117 women (33.2 ± 0.5 yrs. [20–44]) with pGDM underwent an oral (OGTT, 75 g) and intravenous (FSIGT, insulin-modified) glucose tolerance test 8 – 12 weeks after delivery. Women who manifested DM2 were excluded from follow-up (n=8, i.e. 7%).At 1 years's follow-up, 98 women (84%) were reexamined by an OGTT.
**Results:** At baseline, 87 women (74%) had normal glucose tolerance (NGT; 32.6 ± 0.5yrs, [20–44]) and 30 women (26%) had an impaired glucose metabolism (IGM: IFG, IGT or DM2; 34.7 ± 0.9yrs,[20–42]). The sensitivity of ADA-criteria to detect IGM was 30 %, of WHO-criteria 93.3 %. After one year, the prevalence of women displaying NGT or IGT did not change with another 8.5% developping overt diabetes. Since more women had IFG (+2.7%) as well as both, IFG and IGT (+3.1%), ADA-criteria detected 52 %

of all women with IGM, but WHO-criteria detected all women with IGM. 67 pGDM who maintained NGT ($pGDM_N$) in the course of one year had a lower waist circumference ($p < 0.002$), lower plasma concentrations of cholesterol and LDL ($p < 0.0001$, both), whereas adiponectin (AC) did not change. Most glucose parameters, including insulin sensitivity, ameliorated slightly, except for fasting glucose which deteriorated ($p < 0.002$). Among the 9 pGDM who moved from NGT at baseline to IGM within 1 year ($pGDM_I$), no significant changes in body composition or lipid metabolism were observed. Apart from hyperglycemia, insulin secretion increased ($p < 0.03$) and OGIS decreased ($p < 0.04$). Comparing $pGDM_I$ to $pGDM_N$ in their baseline characteristics, $pGDM_I$ already had higher fat mass, waist circumference and plasma leptin ($p < 0.05$) and lower plasma adiponectin ($p < 0.002$). Their insulin secretion secretion parameters were higher ($p < 0.05$) and insulin sensitivity lower ($p < 0.02$). Upon logistic regression analysis with the occurrence of IGM as the dependent variable and all the other parameters as independent variables, only glycemia, stimulated insulin release and insulin sensitivity remained as predictors, explaining 35% of its occurrence.

**Conclusion:** One year after delivery, a total of 15% of pGDM developped overt DM2. One fourth had IGM and were markedly insulin resistant. The glucose and insulin concentration curves during the OGTT and insulin sensitivity can predict the development towards DM2 within a cohort of Caucasian women with a normal glucose tolerance after delivery.

## 1000

**Precocious markers of cardiovascular risk and vascular damage in apparently healthy women with previous gestational diabetes**

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**Background and aims:** Women with previous gestational diabetes mellitus (GDM) have high risk for future type 2 diabetes (T2DM). Insulin resistance (IR) may precede for many years T2DM onset and it is the major feature of metabolic syndrome. Metabolic syndrome (MS) is related to increased risk for cardiovascular diseases. This study aims to identify endothelial dysfunction and several cardiovascular risk factors in women with previous GDM.

**Materials and methods:** A cross-sectional study included 45 non diabetic women, 20 with (group A) and 25 without previous GDM (group B), selected at least one year after delivery. Body mass index (BMI), abdominal circumference, blood pressure, serum creatinine, lipids, liver enzymes, uric acid, nonesterified fatty acids, C-reactive protein and plasma glucose, insulin, fibrinogen and plasminogen activator inhibitor 1 were measured. HOMA IR and β were estimated. Pre and post induced ischemia videocapillaroscopy was performed in hand nailfold to evaluate microvascular morphologic aspect and functional response.

**Results:** Abdominal circumference and fasting glucose were significantly higher in group A (p=0,01 and p=0,002 respectively). Women with BMI<25 kg/m² had significantly higher levels of fasting insulin and HOMA IR than controls (p=0,008 and 0,05 respectively). One or more MS features were found 4,7 times more in group A than B (p=0,03). Abnormal morphologic findings were also more frequent and flat papillae were 3,3 times more prevalent in group A (p=0,003).

**Conclusion:** We conclude that cardiovascular risk factors and abnormalities of microcirculation were significantly increased in young women with previous GDM, obese or not, even without diabetes or abnormal glucose tolerance.

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## 1001

**Association between metabolic syndrome and novel cardiovascular risk factors in women with previous gestational diabetes mellitus**

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**Background and aims:** Women with previous Gestational Diabetes Mellitus (pGDM) are at risk for developing type 2 diabetes and frequently show components of the insulin resistance syndrome that could contribute to cardiovascular risk. Aim of this study was to evaluate the rate of MS and associated novel cardiovascular risk factors in pGDM women.

**Materials and methods:** We studied 166 pGDM women and 98 controls (CON) 16 months after delivery. Fasting plasma glucose (FPG), insulin, lipid profile, fibrinogen, high sensitive C-reactive protein (CRP), omocystein, serum uric acid, blood pressure and anthropometric parameters were determined. HOMA-R was used to estimate insulin resistance and metabolic syndrome (MS) was defined by NECP-ATP III criteria.

**Results:** The two groups were comparable for age (34.7 ± 4.2 vs 33.9 ± 3.9 years), parity (56.2 vs 51.4%), and BMI (25.4 ± 5.4 vs 24.2 ± 4.2 kg/m²). pGDM compared to CON were more insulin-resistant (HOMA-R: 2.18 ± 2 vs 1.35 ± 0.6; p<0.05). The prevalence of MS determinants was higher in pGDM than in CON: high FPG 7.8 vs 0% (p<0.005), low HDL 37.3 vs 17.3% (p<0.01), hypertriglyceridemia (9.63 vs 2%; p<0.01), abdominal obesity (34.3 vs 18.3% ; p<0.01), while high blood pressure values did not differ (5.4 vs 7.1%; ns). The prevalence of MS was 9.03% (n=15) in pGDM and 0.9% (n=1) in CON (p<0.01). Women with MS (MS+, n=16) compared to those without MS (MS-, n=248) showed significantly higher fibrinogen (348.7 ± 54 vs 311.4 ± 70 mg/dl p<0.04), serum uric acid (4.7 ± 1.2 vs 4 ± 0.8 mg/dl p<0.01), CRP (6.1 ± 5.03 vs 1.7 ± 2.3 mg/dl p<0,001), HOMA-R (4.1 ± 2.3 vs 1.8 ± 1.1; p=3 criteria 6.1 ± 5 mg/dl p<0.0001). In addiction CRP levels were positively correlated to FPG (r2=0.041, p<0.01) and insulin (r2=0.21, p <0.001).

**Conclusion:** This study shows that MS is present in a sizable proportion in pGDM women and is associated with novel cardiovascular risk factors (fibrinogen, serum uric acid and CRP). Levels of CRP tend to increase accordingly to the number of determinants of MS. The identification of MS in women with pGDM should be extensively sought in order to reduce the impact of cardiovascular risk.

## 1002

**Characteristics of women with GDM who do not appear for glucose re-evaluation post-partum**

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**Background and aims:** In an earlier study we found that a significant percentage of previous GDM women developed glucose intolerance (14%), or DM2 (5.6%) up to 10 years post partum. The most important independent predictors for future DM2 development were fasting and 2 h glucose values in the diagnostic OGTT 100g during pregnancy. These findings were based on the relatively smaller proportion of GDM women who show up post partum for reevaluation. Therefore, the aim of this study is to (a) identify whether previous GDM women who are not followed up have the same risk profile for DM2 with respect to pathophysiological characteristics and (b) to establish any socioeconomic parameters which may influence their non-attendance.

**Materials and methods:** Of the 1079 women diagnosed with GDM between 1991–2002, 597 revisited post partum for metabolic evaluation (follow-up group 34.9%), while 1112 did not revisit (non-follow-up group 65.1%). All GDM women were appropriately advised to return for reevaluation 2 months after delivery or upon cessation of breast-feeding. In all women during pregnancy the following parameters were reported: age, education status, smoking, family history (FH) for DM2, birthweight, pre-pregnancy BMI, blood pressure (BP), gestational age (GA) when the diagnostic OGTT-100g was performed, glucose and insulin plasma levels during the OGTT and $HbA_{1c}$. For statistical analysis t-test and $\chi^2$ were used.

**Results:** There was no difference between the follow-up and the non-follow-up groups with regard to demographic and pathophysiological features such as age BMI, BP, FH and glucose and insulin levels in OGTT and $HbA_{1c}$. However, differences were found with regard to educational status, as of the women with higher education 41.5% appeared for follow-up, compared to 33.3% of the women with secondary and 25.7% of the women with compulsory education (p<0.001). Further, women of the follow-up group reported one week earlier for GDM screening during pregnancy (GA 26 ± 7 vs 27 ± 7wks p<0.001) and smoked significantly less during pregnancy (11.1% vs 16.5% p<0.01). Finally GDM women who were treated with insulin during pregnancy revisited to a significantly greater extent compared to those treated only with diet (40.7% vs. 33. 3% p<0.001).

**Conclusion:** There was no difference in pathophysiological features during pregnancy between ex-GDM women who returned post-partum for reevaluation, compared to those who did not. Hence, women in both groups are at the same risk for glucose intolerance after delivery. Therefore, consistent strategies will have to be developed to persuade GDM women of the need for postpartum evaluation. As educational status appears to play a role in behavior, the lower their education level the greater the effort must be.

## 1003

**The effect of the gestational diabetes mellitus control on the health of newborns**

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**Background and aims:** Diabetes mellitus in pregnancy is associated with considerably higher incidence of maternal, fetal and neonatal complications. The aim of the study was to assess the effect of the control of gestational diabetes mellitus on the health status of the newborns expressed as body length, weight and Apgar score.
**Materials and methods:** Two groups of pregnant women with confirmed gestational diabetes mellitus were compared. The first group (n=179) was treated by diabetic diet only, the second group (n= 42) was treated by insulin because of high postprandial glucose levels (the mean time of starting insulin therapy was 31$^{th}$ (± 5,6) week, the mean insulin dose was 17,4 ± 13,1 IU/day). Women in both groups were of similar age and body mass index.
**Results:** At the time of diagnosis and in the 35$^{th}$ week of pregnancy, women treated by insulin had significantly higher level of glycated hemoglobin (HbA1 c) (calibration according to DCCT) than women treated by diet. However the level of HbA1 c was in physiological range in both groups. Women treated by insulin had significantly higher fasting and postprandial glucose levels (Table).
Newborns of women treated by insulin had significantly lower Apgar score in the first and fifth minute after delivery than newborns of women treated by diet. No differences in the weight and length of the newborns between both groups were found (Table).

| | Diet | Insulin therapy | P |
|---|---|---|---|
| Mean age | 30,1 ± 4,7 years | 31,0 ± 5,6 years | n.s. |
| BMI before pregnancy | 24,8 ± 3,9 | 24,9 ± 4,9 | n.s. |
| BMI increase in time of diabetes diagnosis | 3,6 ± 1,6 | 2,6 ± 1,3 | 0,001 |
| Time of diagnosis (number of week of pregnancy) | 29,7 ± 4,4 week | 26,1 ± 5,6 week | < 0,001 |
| HbA1 c in 35th week of pregnancy | 4,6 ± 0,4 % | 5,6 ± 0,7 % | < 0,001 |
| Mean fasting glycemia in 35th week of pregnancy | 4,5 ± 0,5 mmol/l | 5,7 ± 1,0 mmol/l | < 0,001 |
| Mean postprandial glycemia in 35th week of pregnancy | 6,1 ± 0,8 mmol/l | 8,4 ± 1,2 mmol/l | < 0,001 |
| Newborn weight | 3367 ± 490 g | 3353 ± 617 g | 0,8 |
| Newborn lenght | 53,9 ± 2,3 cm | 49,5 ± 2,5 cm | 0,05 |
| Apgar score 1st minute | 9,0 ± 1,3 | 7,4 ± 2,3 | < 0,001 |
| Apgar score 5th minute | 9,7 ± 0,7 | 8,7 ± 1,5 | < 0,001 |

**Conclusion:** Postprandial glycemia and HbA1 c could be of extremely importance for well being of the newborns of women with gestational diabetes mellitus. Therefore very tight control of these parameters (even if they are almost in the physiological range) is warranted and the rule should be the lower the better.

## 1004

**Overt diabetes mellitus as a remote complication in women after gestational diabetes (GDM)**

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**Background and aims:** To examine factors associated with the risk for developing post partum diabetes mellitus in women with GDM in previous pregnancy
**Materials and methods:** 79 women with diabetes mellitus who has been treated in our Department during the last 10 years because of gestational diabetes mellitus were enrolled into the study. A retrospective study was performed using our computer database, to answer the question which maternal and fetal factors present during pregnancy complicated with GDM predict type 2 (DM2) and type 1 (DM1) diabetes.
**Results:** In the study group 38 (24,7%) patients developed type 1 and 36 (23,4%) developed type 2 diabetes. Five of them (3,25%) manifested impaired glucose tolerance. In both groups we have analyzed patients age, when diabetes developed (26,5 ± 4,7 years in type 1 DM and 32,9 ± 4,9 years in type 2 DM respectively, p<0,0001), time after GDM, when DM developed (1,8 ± 1,3 year in DM1 and 4,4 ± 4,0 in DM2, p<0005), week of pregnancy when GDM was diagnosed (24,6 ± 11,1 in DM1 and 27,3 ± 8,8 weeks in DM2, p=NS), age of patients in pregnancy with diagnosed GDM (25,2 ± 4,4 in DM1 and 28,5 ± 4,5 years, p<0,005). We also analyzed anthropometrical parameters in the study group. The BMI at the beginning of index pregnancy was significantly different between patients with subsequently developed DM1 and DM2 (23,3 ± 4,2 and 30,1 ± 6,1 kg/m$^2$, respectively). Fasting glucose levels at the time of GDM recognition differ significantly these two subgroups (204 ± 110,9 mg/dl in DM1 and 130,0 ± 41,0 mg/dl in DM2, p<0,002). Two hours OGTT levels and $HbA_{1C}$ at the time of GDM diagnosis and in the third trimester were not significantly different between groups.
Multivariate regression analysis has shown that diabetes onset after GDM was significantly related to the number of pregnancies, in which GDM had developed, both values of OGTT, maternal BMI before pregnancy and the weight gain during pregnancy, but neither maternal $HbA_{1C}$ nor birth weight of the offspring correlated with subsequent diabetes developing.
To look for factors predictive for both types of DM, logistic regression analysis was performed. The strongest relation to the development of DM1 was found for fasting OGTT, age at DM1 onset, age at the time of GDM presence, and prepregancy BMI, but no relation was found for number of pregnancy before GDM developed and with gestational age at the time of GDM recognition. DM2 was strongly predicted with prepregancy BMI, with the age of patients, and weaker but still significantly with fasting OGTT, and age of patients when GDM developed.
**Conclusion:** Obesity, elevated fasting glucose levels and young age of mother at the time of GDM development seem to be the strongest factors predictive for diabetes mellitus later in life. More active strategies for future weight control and life style advice after delivery might therefore be indicated for women with GDM.

## 1005

**Glucose intolerance following gestational diabetes mellitus in a multi-ethnic population**

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**Introduction:** Gestational Diabetes Mellitus (GDM) predisposes to future Type 2 Diabetes Mellitus (T2DM), this risk being increased within the Indo-Asian population. Repeat screening with a 75g Oral Glucose Tolerance Test (OGTT) is recommended 6 weeks post delivery to establish which women have impaired fasting glycaemia or impaired glucose tolerance (IGT), and T2DM.
**Method:** We reviewed the notes of 264 women who developed GDM between 1999–2003, using Euro-king and Apex electronic databases. The sample ethnicity reflected the local population of Indo-Asian and Caucasian women (36% vs 64%). The performance of a post-natal OGTT was identified and the results documented. Further analysis then compared the results to a similar study cohort (1995–1999)
**Results:** 194 women (73%) had a post-natal OGTT undertaken, concordance with screening being similar in both groups (78% of Indo-Asian and 71% of Caucasian women).
Thirty-seven women (19%) had impaired glucose tolerance, 18 (48%) being Indo-Asian origin and 19 (52%) Caucasian. This represented 19% Indo-Asian women, and 16% Caucasian women.
An additional 19 (9.7%) women were diagnosed with T2DM, 12 (63%) being Indo-Asian and 7 (37%) Caucasian. This reflects an increased prevalence of T2DM in Indo-Asian women post GDM (12.5% vs. 6%).
The uptake of the post-natal OGTT has improved compared to the earlier cohort (73% vs 44%). However, the prevalence of T2DM and IGT has remained similar in both cohorts (11.8% vs 9.7%) and (17.7% vs 19%).
**Conclusion:** The increased uptake has resulted in more women being identified at an earlier stage with glucose intolerance, and the potential to reduce future complications. More Indo-Asian women have persistent post-natal glucose intolerance, and this is in keeping with a more rapidly progressive disease process.

## 1006

**Pregnancy planning and morbidity in pregnancies complicated by pre-gestational diabetes mellitus**

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**Background and aims:** A good metabolic control and medical care before and during pregnancy are very important in order to reduce obstetric morbility in women with pregestational diabetes mellitus (PDM). The present study was conducted with the aims to asses the impact of pregnancy planning and chronic diseases on the rate of delivery by Caesarean section, Miscarriage, Voluntary Abortion, Major Malformations (MM), Macrosomia and Premature Delivery (PD).
**Materials and methods:** 308 women, 229 (75,4%) with diabetes mellitus type 1 (DM1), 77 (23,9%) with diabetes mellitus type 2 (DM2) and 2 (0,7%) with abnormal glucose tolerance (AGT). The average age of the group was 30.6 ± 11.46 years, and the average age per groups was 28.8 ± 4.8, 35.5 ± 5.41 and 43.5 ± 0.5 years in patients with DM 1, DM 2 and AGT. The average age when the disease appeared was 16.8 ± 8.36, 29.5 ± 6.3 and 40 years respectively.
As regards chronic complications: 18.5% presented retinopathy to some extent, 6.8% nephropathy and 1.9% neuropathy.
**Results:** 39,5% of pregnancies were planned (PP) and 60,5% were not planned (NPP). Amongst patients with DM1 there was a 45% rate of PP and in patients with DM type 2 the rate was 19,5% (p≤0,001).

HbA1 c prior to pregnancy and each 4 weeks were:

| Hba1c | Prior | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PP | 6,1 | 6,26 | 5,97 | 5,67 | 5,63 | 5,55 | 5,59 | 5,69 | 5,70 | 5,58 | 5,23 |
| NPP | 8,29 | 7,76 | 6,79 | 6,1 | 5,29 | 5,86 | 6,01 | 5,91 | 6,16 | 5,83 | 5,55 |

Using 6,5% and 5,5% as a break point for HbA1 c, prior and during pregnancy respectively, significant differences were observed between PP´s and NPP´s before and during weeks 8, 12 and 16 (p≤o,oool, p≤o,o65, p≤o,o5 respectively).

Obstetric morbility was as follows:

| | Caesarean section | Miscarriage* | Voluntary Abortion | MM** | Macrosomia PD*** | |
|---|---|---|---|---|---|---|
| PP | 43 (14%) | 13 (4,2%) | 0 | 2 (0,7%) | 13 (4,2%) | 21 (6,8%) |
| NPP | 63 (20,4%) | 33 (10,7%) | 7 (2,2%) | 10 (3,2%) | 20 (6,5%) | 40 (13%) |

* p≤0,14 ** p≤0,098 *** p≤0,121
RR=0,597 RR=0,29 RR=0,617
As regards chronic complications for diabetes, it was observed that the presence of retinopathy in the PP group was associated with more frequent delivery by caesarean section (p≤0,06; RR=2,31).
**Conclusion:**
1. The number of planned pregnancies is low, especially in patients with DM2.
2. A better metabolic control prior to pregnancy and until week 16 was achieved thanks to pregnancy planning, which resulted in a significant lower rate of miscarriage, Major Malformations, and Premature Delivery.
3. Caesarean section and voluntary abortion were slightly more frequent in NPP
4. The presence of diabetic retinopathy was associated with a higher rate of caesarean delivery rate.

# PS 97

# Young people with Type 1 diabetes

## 1007

**Is the overestimation of the recorded diagnosis of diabetic ketoacidosis a reflection of a true clinical problem?**

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**Background and aims:** Diabetic ketoacidosis (DKA) is a potentially life threatening complication of diabetes. The diagnosis based on clinical and biochemical parameters, which must include demonstration of ketosis. A benchmark for auditing the quality of diabetic services in the UK is DKA incidence within a hospital's catchment area. This data is dependent on entries in medical notes, which are processed by non-medical coders who assign an ICD 10 diagnostic code. Errors can occur because of inaccurate diagnosis by medical practioners or inaccurate interpretation of medical entries by coders. Inaccuracies by medical practioners can lead not only to coding errors but also to inappropriate management
**Methods:** We studied patients with a diagnostic coding of DKA, to determine whether the coded diagnosis was accurate, at what level of the process any errors were occurring and if these were leading to inappropriate management. All episodes coded as DKA between 1998–2003 were identified from our hospital database and a random sample of case-notes were analysed retrospectively to see whether they fulfilled the diagnostic criteria defined by the American Diabetes Association. 75/440 (17%) episodes were analysed.
**Results:** 25 (33.3%) episodes were true DKA, 43 (57.3%) had nonketoacidotic hyperglycaemia, 2(2.7%) had hypoglycaemia, 2 (2.7%) did not have diabetes and 3 (4.0%) had insufficient data. In the 50 episodes that did not fit the diagnostic criteria for DKA, 17 (34%) had a wrong diagnosis of DKA made by the admitting doctor, whilst the remainder were miscoded by coders. Of these 17 episodes misdiagnosed by medical staff, 9 (53%) had significant ketonuria without acidosis, 7 (41%) had absent or insignificant ketonuria and 1 (6%) had no record of urinanalysis but did not have acidosis. 13 (76%) were however managed with sliding scale insulin. 7/33 (21%) episodes miscoded by coders had also been treated by sliding scale insulin.
**Conclusion:** This audit suggests that current systems over-estimate episodes of DKA by 3-fold. Some of this error appears to be due to inaccurate transcription of the true diagnosis to a corresponding ICD 10 code. However a large number of patients were inaccurately diagnosed with DKA by medical staff, which frequently led to inappropriate management such as unnecessary admission and intravenous insulin sliding scales. Our data imply that by extrapolation, up to 117 episodes over 5 years may have been inappropriately managed with intravenous insulin sliding scales. The use of capillary blood ketone testing has been shown to be a more specific test for ketoacidosis than urinanalysis, can be done at the bedside, and may be a useful adjunct to help distinguish DKA from uncomplicated hyperglycaemia. We have altered the coding system to avoid transcription of inaccurate ICD codes, and propose to introduce a pilot study to assess whether near patient testing for ketonaemia improves diagnostic accuracy.

## 1008

**Increase in risk of diabetic ketoacidosis in minority children is due to modifiable risk factors**

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**Background and aims:** Diabetic ketoacidosis (DKA) and severe hypoglycemia (SH) are major preventable acute complications of type 1 diabetes. We assessed the incidence and risk factors for DKA and SH in a population sample including major U.S. ethnic groups.
**Materials and methods:** 1,151 type 1 diabetic children, aged 0–19 years, residing in the Denver Metropolitan Area, and seen at the Barbara Davis Center for Childhood Diabetes during 1996–2000 were followed for an average of 3.2 (± 1.7 SD) years. Of those, 10% were Hispanic, 4% African American, 2% Asian American, and 84% non-Hispanic white (NHW). DKA (emergency department visit and/or hospital admission) and SH (loss of consciousness, seizure, and/or admission) were identified prospectively.
**Results:** There were 297 DKA episodes and 726 SH episodes in the study cohort during 3,707 p-yrs of follow-up. The incidence of DKA was two-fold

higher (p<0.02) in the minority patients (15.4/100 p-yrs), compared to NHW (6.8). The incidence of SH was comparable in both groups (19.4 vs. 20.7). Poisson regression analysis was stratified by age (<13 vs. ≥13) and sex-adjusted. In younger children, the risk of DKA increased with an increase in HbA1 c level (RR=1.67 /1%; 95% CI 1.42–1.96) and with higher reported insulin dose (1.37/0.2 U/kg; 1.17–1.67), but did not differ by ethnicity (1.17; 0.73–1.88) after adjusting for other risk factors. In older children, DKA risk increased with higher HbA1 c level (1.39/1%; 1.25–1.54), higher reported insulin dose (1.15/0.2 U/kg), underinsurance (2.06; 1.51–2.81), and psychiatric disorders (RR=1.69; 0.96– 2.71 in males and 3.17; 2.21–4.55 in females), but also did not differ by ethnicity (1.33; 0.92–1.92). Ethnicity did not affect the risk of SH, adjusting for diabetes duration, underinsurance, HbA1 c, and psychiatric disorders.
**Conclusions:** Minority children in the U.S. are at a high risk of DKA due to modifiable risk factors such as higher HbA1 c level, higher reported insulin dose, underinsurance, and psychiatric disorders. Minority children should be targeted with interventions including lowering financial barriers to care, tighter glycemic control, and aggressive treatment of co-morbid conditions.

## 1009

**The association of chemokine RANTES levels with insulin administration during the first year of Type 1 diabetes mellitus in children**
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**The aim** of the study was to estimate the level of RANTES (Regulated on Activation, Normal T cell Expressed and Secreted) in relation to residual insulin secretion during the first year of type 1 diabetes.
**Materials and methods:** 99 type 1 diabetic patients (48 females, 51 males), aged 1–18.2 years at diagnosis (mean age 10.2 ± 4.01) participated in the study. The level of RANETS was measured by ELISA. The C-peptide level at the diagnosis and after 1, 2, 3, 6 and 12 months of the disease was estimated by radioimmunoassay. The daily insulin dose during the first year of the disease was also evaluated.
**Results:** The C-peptide level was significantly higher in the second month of the disease compared to the levels at the diabetes onset and after the first year of the disease (p<0.001). Similarly, the daily insulin dose was the lowest in the second month of the treatment (p<0.01); this corresponded to the average time of "honey moon" appearance. There was no correlation between RANTES level and sex. No correlation was found between RANTES level and the age at the diabetes diagnosis, either. The performed analysis revealed a positive correlation between RANTES level and daily insulin dose at the diabetes onset (r=0.5; p<0.05). During the first year of the disease transitional increase in insulin secretion was observed.
**Conclusion:** During the first year after type 1 diabetes diagnosis a transitional increase in insulin secretion and decrease in insulin requirement is observed. The insulin requirement at the diagnosis of type 1 diabetes may be related to the activity of inflammatory reaction of Langerhans islets, the marker of which may be the level of the chemotactic cytokine - CCL5/RANTES.
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## 1010

**Disturbances in «growth hormone – insulin-like growth factor» axis in young patients with DM Type 1**
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**Background and aims:** Our research was aimed at defining IGF-1 and IGFBP-3 levels for assessing the expressiveness of IGF-1/IGFBP axis deviation of in young persons with DM 1.
**Materials and methods:** The study was carried out on 114 young patients with DM 1 (60 female and 54 male patients), mean age 20,6 ± 0,59 yrs. The clinical characteristic of the surveyed patients with DM 1 are as follows: mean age at the moment of DM 1 manifestation - 13,15 ± 0,62 yrs, duration of disease - 7,05 ± 0,51 yrs, daily mean insulin doze - 0,85 ± 0,02 U per 1 kg body weight. The control group consisted of 37 practically healthy persons, sex and age matched.
IGF-1 and IGFBP-3 levels were measured by the immunoenzymatic method.
**Results:** IGF-1 level median in young patients with DM 1 was significantly lower than in control group (85,1 ng/ml vs 219,6 ng/ml, p<0,000001). IGFBP-3 level median in DM 1 patients was also lower than in control group, but the change was not that statistically significant (1678,9 ng/ml vs 1965,3 ng/ml, p=0,058). IGF-1 level median in young patients with DM 1 is significantly lower in female patients compared with male patients (57,9 ng/ml vs 101,1 ng/ml, p=0,034), whereas control group did not demonstrate similar correlation. IGFBP-3 levels both in control group and in DM 1 patients had a tendency to reduce in female patients. In both female and male DM 1 patients IGF-1 level median was significantly lower than in control group (57,9 ng/ml vs 192,9 ng/m, p=0,00001 and 101,1 ng/ml vs 236,01 ng/ml, p=0,0002 respectively).
We noted a significant reduction of IGF-1 levels in patients with longer diabetes duration (112,1 ng\ml for duration < 5 years vs 78,6 ng/ml for duration > 5 years, p=0,033). A tendency to IGFBP-3 levels reduction with longer diabetes duration was also revealed. At the same time daily mean insulin dose per 1 kg body weight significantly increased in patients with longer duration of DM 1 (0,82 U/kg/day vs 0,94 U/kg/day, p=0,0003).
**Conclusion:** Decreased IGF-1 and IGFBP-3 levels are noted in DM 1 young patients compared with control group. Gender differences of IGF-1 levels were observed: IGF-1 levels were significantly lower in DM 1 females. In patients with longer diabetes duration IGF-1 reduction degree increases. This GH/IGF-1/IGFBP-3 axis disturbance may indirectly point at the increase of the daily mean insulin dose per 1 kg body weight.

## 1011

**Neuropsychological and brain metabolite profiles of children with Type 1 diabetes 12 years after disease onset**
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**Background and aims:** To describe neuropsychological and brain metabolite profiles in children with type 1 diabetes twelve years after disease onset.
**Materials and method:** Children with type 1 diabetes who had previously been assessed soon after diagnosis, and again two and six years later, were re-evaluated 12 years after disease onset. Results were compared to a community control group that was assessed at similar times. All participants underwent an MRS study and completed a battery of neuropsychological measures.
**Results:** Twelve years after disease onset, children with type 1 diabetes performed more poorly than control subjects on Performance IQ (PIQ) and measures of intelligence, attention and executive functions. MRS data indicate higher levels of myoinositol (p<0.01) and choline containing compounds (p<0.01) in the frontal lobes, compared to controls. In basal ganglia, total NA was reduced in children with type 1 diabetes (p<0.01).
**Conclusions:** MRS and neuropsychological profiles of children with type 1 diabetes 12 years after disease onset are consistent with subtle changes in anterior brain regions. Increased levels of myoinositol may be a marker of fluid imbalance resulting from regular disruption of glucose homeostasis. Myoinositol is also associated with increased gliosis. As glial cells contain a small store of glycogen, increased myoinositol may reflect a mechanism to protect the brain during hypoglycaemia. Increased levels of choline containing compounds suggests altered membrane turnover. Reduced NA in basal ganglia suggests reduced neuronal population or function in children with type 1 diabetes.

Significant group differences on neuropsychological measures

| | Visuo-spatial reasoning | PIQ | Concept formation | Attentional switching | Selective Attention |
|---|---|---|---|---|---|
| IDDM (n=25) | 51.9 (9.95) | 100.8 (11.4) | 8.88 (1.7) | 9.54 (3.0) | 7.08 (2.86) |
| Control (n=35) | 58.2 (7.8) | 109.7 (11.8) | 10.2 (1.95) | 11.17 (2.85) | 9.83 (3.19) |

*This research has been conducted with the financial support of Juvenile Diabetes Research Foundation.*

## 1012

**Therapeutic strategies, treatment satisfaction and glycaemic control in adolescent patients with Type 1 Diabetes. Report on the German Multicentre Adolescent Diabetes (MAD) study**

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**Background and aims:** For paediatric patients with Type 1 diabetes, adolescence and puberty are arguably the most difficult time in life to achieve good metabolic control. Therefore, we performed a cross-sectional multicentre study to analyse the different strategies of insulin therapy on treatment acceptance in patients, and the effect on glycaemic control.
**Materials and methods:** The Multicentre Adolescent Diabetes (MAD) observational study was carried out in seven paediatric diabetes centres, each treating >100 patients. For 3 months, clinical data, $HbA_{1c}$ (using the same assay in all centres) and treatment satisfaction using the Diabetes Treatment Satisfaction Questionnaire (DTSQ) were recorded in adolescent patients (11–18 years).
**Results:** A total of 840 patients (49.8% female; age 14.6 ± 2.0 years; diabetes duration 5.9 ± 3.8 years) were eligible. Mean $HbA_{1c}$ was 7.8 ± 1.5% with significant centre differences and higher values in patients with a longer duration of diabetes, with no gender effect. The incidence of severe hypoglycaemia was 14.1 episodes/100 patient-years. Insulin glargine (LANTUS®) was the most frequently used basal insulin (n=274), followed by NPH insulin (n=229), NPH insulin plus semilente (n=215), and continuous subcutaneous insulin infusion (CSII) (n=122). Basal insulin comprised 41 ± 13% of the daily insulin dose. Rapid-acting insulin analogues were taken by 52% of patients; 45.5% took greater than 4 daily injections. Treatment satisfaction was high: 4.4% (37) patients scored the maximum of 36; 39.2% (329) scored 31–36.
**Conclusion:** New therapeutic strategies including rapid-acting prandial insulin and basal insulin glargine are widely used in adolescent patients. The introduction of modern treatment options may increasingly influence the improvement in treatment satisfaction, rate of hypoglycaemia, and glycaemic control, all of which are traditionally difficult to achieve in this age group.
*Supported by: Aventis Pharma.*

# PS 98

# Somatic neuropathy

## 1013

**Nerve conduction velocity is independently related to diabetic complications and risk factors for microvascular disease in Type 1 diabetes**

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**Background and aims:** The principal determinant for the development of diabetic neuropathy is glycaemic control ($HbA_{1c}$ and duration of diabetes). However, other determinants of microvascular disease, notably blood pressure, are also thought to play a role.
**Materials and methods:** We cross-sectionally studied the relation between nerve conduction velocities (NCV), the presence of diabetic complications and microvascular risk factors in 503 participants (246 men and 257 women) of the EURODIAB Prospective Complications Study (EPCS) of type 1 Diabetes. Data were collected in 13 centres in 9 European countries. NCVs were measured in the ulnar and sural sensory nerves and the peroneal motor nerve according to a standard protocol. Results for the three nerves were summarised per individual in a summed Z-score.
**Results:** Men had lower NCV scores compared to women, indicating poorer nerve conduction (p=0.003). NCV scores were further inversely related to age, $HbA_{1c}$ and duration of diabetes. After adjustment for sex, $HbA_{1c}$, duration of diabetes and study centre, NCV scores were related at a statistically significant level to systolic blood pressure, height, albumin excretion rate and the presence of retinopathy. With the same adjustments, those in the highest quintile of the NCV score had a 53.9% (95% CI: 44.5;63.4) prevalence of retinopathy and a 11.7% (3.9;19.6) prevalence of nephropathy, compared to 80.8% (69.7;92.0) and 31.7% (23.3;40.1) respectively in the lowest NCV quintile (p-values for trend: p< 0.001 for both nephropathy and retinopathy).
**Conclusion:** Our results show that nerve conduction, measured by electrophysiology is related to the presence of hypertension, and microvascular diabetic complications independently of age, sex, HbA1 c and duration of diabetes. These results are in accordance with the microvascular pathogenesis hypothesis of diabetic neuropathy.
*Supported by: European Union, Pfizer Inc.*

## 1014

**Baroreflex sensitivity in overweight subjects and Type 2 diabetic patients**

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**Background and aims:** Previous studies suggest that baroreflex sensitivity (BRS) is impaired early in type 2 diabetes (T2D) and may be altered in obesity. The aim of the present study was to compare BRS in T2D and non-diabetic (ND) subjects with or without overweight and to examine the determinants of BRS in these groups.
**Materials and methods:** Seventy-nine subjects (20–65 years old), 34 T2D patients (16M/18F; 22–42 $kg/m^2$) were compared to 45 ND subjects (19M/26F; 20–50 $kg/m^2$). Blood pressure was recorded during 6 min at controlled breathing rate (12 cycles/min) in supine and standing position using a Finapres device. Pulse interval (PI) and systolic blood pressure (SBP) variability were quantified on 2048-point stationary time series, corresponding to a period of 204.8 s after resampling at 10 Hz. Frequency domain measures were obtained using a Fast Fourier Transform. The low frequency (LF) component was obtained by integration of the values of the consecutive bands from 0.05 to 0.150 Hz (LF range). Spontaneous BRS was evaluated by a cross spectral method, ie by the gain of the transfer function between SBP and PI in the LF range. The transfer function analysis was applied to assess the relationship between spontaneous SBP and PI oscillations. This procedure provides values of gain in the frequency domain. The gain defined the ratio between changes in PI and SBP and had units of ms/mmHg.
**Results:** LF-PI and baroreflex gain were significantly lower in T2D patients than in ND subjects (163.8 ± 42.3 vs 455.3 ± 115.1; P < 0.05 and 1.86 ± 0.22 vs 2.74 ± 0.28; P < 0.05, respectively). In ND subjects, and in standing posi-

tion, BMI was significantly and negatively correlated to LF-SBP ($r = -0.45$; $P < 0.01$), LF-PI ($r = -0.48$; $P < 0.001$) and BRS ($r = -0.54$; $P < 0.001$). Likewise, age significantly and negatively correlated to LF-PI ($r = -0.44$; $P < 0.01$) and BRS ($r = -0.56$; $P < 0.001$). A multiple linear analysis indicates that BMI and age explained 50 % of the variance of BRS. These effects were not observed in T2D patients.
**Conclusion:** The present results indicate that BRS is reduced more in T2D patients than in ND subjects and that both body weight and age may contribute to the reduction in BRS.

## 1015

**Evaluation of the new indicator plaster (Neuropad®) in the diagnosis of peripheral neuropathy among Type 2 diabetic patients**

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**Background and aims:** Autonomic sudomotor neuropathy is associated with reduction of plantar sweating and contributes to the pathogenesis of diabetic foot ulcers. Early diagnosis of the sudomotor component of peripheral neuropathy may contribute to detection of patients at high risk for diabetic foot complications. Therefore, the aim of the present study was to evaluate the new indicator plaster (Neuropad®) in the diagnosis of peripheral neuropathy among type 2 diabetic patients.
**Materials and methods:** This study included 104 type 2 diabetic patients (51 men) with a mean age of 64,2 ± 5,6 years and a mean diabetes duration of 12,8 ± 3,7 years. The control group comprised 20 healthy young volunteers (<40 years old). Peripheral neuropathy was diagnosed by means of the Diabetic Neuropathy Index (DNI, normal values: 0–2). Indicator plasters were applied to both soles of patients. Autonomic neuropathy was assessed by means of colour change in the indicator plasters (normal response: colour change within 10 minutes).
**Results:** Peripheral neuropathy was diagnosed in 71 patients (68,27%). Colour change of the plaster in the right sole was associated with colour change in the left sole (p=0,0001). Autonomic neuropathy was diagnosed in 67 patients (94,36%) with peripheral neuropathy and in 10 patients (30,3%) without peripheral neuropathy (p=0,0001). Compared with DNI, sensitivity of the indicator plaster for diagnosing peripheral neuropathy was 94,36% and specificity was 69,69%. Overall prevalence of neuropathy was higher using the indicator plaster (77 patients, 74,04%) than using the DNI (71 patients, 68,27%). Colour change of the indicator plaster was completed within 10 minutes in 19 volunteers (95%). Time until complete colour change of the indicator plaster was 23,80 ± 6,7 minutes in patients with peripheral neuropathy and 7,67 ± 1,22 minutes in patients without peripheral neuropathy (p=0,001). Among patients with peripheral neuropathy, time until complete colour change of the indicator plaster was 14,20 ± 1,9 minutes in those with a DNI value between 2,5 and 4,5, while it was 32,8 ± 2,6 minutes in those with a DNI value between 5 and 8 (p=0,003).
**Conclusions:** Use of the new indicator plaster has a very high sensitivity in detection of diabetic peripheral neuropathy. Autonomic sudomotor dysfunction can even be demonstrated in a considerable part of patients with normal DNI. Therefore, the new indicator plaster may prove useful in detection of patients at high risk for diabetic foot complications. Finally, time until complete colour change of the indicator plaster is associated with severity of peripheral neuropathy.

## 1016

**Positive sensory symptoms of diabetic peripheral neuropathy are associated with poorer functional status and lower quality of life in patients with a non-positive Semmes-Weinstein 5.07 monofilament**

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**Background and aims:** Diabetic peripheral neuropathy (DPN) is a common and debilitating consequence of diabetes mellitus. Current clinical guidelines recommend using the Semmes-Weinstein 5.07 (10-g) monofilament test to identify an insensitive foot at risk for ulceration. Although this test identifies loss of protective sensation, it does not address other symptoms of DPN, such as burning, tingling and allodynia. The objective of this analysis was to document, in patients who did not have an insensitive foot at risk for ulceration using the S-W monofilament test, the relationship between worsening positive sensory symptoms of DPN and functional health status and quality of life.
**Materials and methods:** We randomly selected 4,000 currently enrolled members from the diabetes registry maintained by Kaiser Permanente Northwest, a 450,000-member non-profit HMO, to receive a questionnaire that contained self-administered versions of the Neuropathy Total Symptom Score-6 (NTSS-6-SA), a validated instrument designed to identify the positive sensory symptoms characteristic of DPN; the Short-Form 12 Health Survey (SF-12); the EuroQol (EQ-5D) quality of life instrument; and the 8-item Patient Health Questionnaire (PHQ-8) depression diagnostic instrument. Two mailings yielded a 61% response rate. We then recruited a subset of respondents to a clinic visit during which the NTSS-6-SA was re-administered and the S-W monofilament test was performed. Of these 397 patients, 281 (71%) reported sensation in all eight sites tested (first, third and fifth toes, and the top of the midfoot). After categorizing these 281 patients with non-positive S-W monofilament tests into three groups based on NTSS-6 categories (no symptoms of DPN, mild occasional symptoms, or moderate to severe frequent symptoms), we compared health status and quality of life across the three groups.
**Results:** Subjects averaged 65.8 years of age and had had diabetes for a mean of 7 years; 55.7% were female. These characteristics did not differ across NTSS-6 categories. We observed a substantial and statistically significant graded association between severity and frequency of positive sensory symptoms of DPN and poorer self-reported health status and reduced quality of life.

| | No DPN Symptoms (NTSS-6 = 0) | Occasional, Mild DPN Symptoms (NTSS-6 ≤ 6) | Frequent, Moderate/Severe DPN Symptoms (NTSS-6 score > 6) | Total |
|---|---|---|---|---|
| Number of Respondents | 62 | 107 | 112 | 281 |
| Mean SF-12 Physical Component | 48.0 | 43.5 | 32.6 | 40.1 |
| Mean SF-12 Mental Component | 54.9 | 54.3 | 49.5 | 52.5 |
| Mean EQ-5D Health Index | 0.88 | 0.81 | 0.61 | 0.74 |

**Conclusion:** Our results confirm that the positive sensory symptoms of DPN severely and negatively affect health status and quality of life. Importantly, these substantial losses occur before, or independently of the neurologic damage that is detected by the predominantly used clinical test, the Semmes-Weinstein 5.07 (10-g) monofilament.

## 1017

**Peripheral neuropathy, glycaemic control and sex in a diabetes clinic with diverse ethnic mix**

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**Background and aims:** Patients with peripheral neuropathy are at greater risk of developing foot ulceration compared to patients without neuropathy. We therefore investigated the prevalence of peripheral neuropathy and distribution among different ethnic groups in a diabetic clinic with a diverse ethnic mix.
**Materials and methods:** The notes of 200 (94F, 106M) consecutive patients attending a general diabetic clinic were reviewed. All patients had type 2 diabetes. The 200 patients comprised 107 (54%) Caucasians, 53 (27%) Asians, 22 (11%) Afro-Caribbeans and 18 (9%) patients of Mediterranean origin (predominantly Turkish).
**Results:** 31% (62; 26F, 36M) patients had clinical evidence (tuning fork +/- biothesiometer readings) of peripheral neuropathy. Patients with neuropathy had significantly worse HbA1 c results than patients without neuropathy [median(range); 9.5% (5.6–13.8) vs 8.3 (5.8–14.1%); p<0.02]. Peripheral neuropathy was present in 29 (27%) Caucasians, 16 (30%) Asians, 11 (50%) Afro-Caribbeans and 6 (33%) patients of Mediterranean origin. The median (range) HbA1 c in these groups was 7.7% (5.6–11.3%) in Caucasians, 8.1% (6.1–13.8%) in patients of Mediterranean origin.58% (15/26) women (particularly Turkish) with neuropathy had an HbA1 c outside the normal range (4.1 - 6.5%), compared to men (23/36; 30%).
**Conclusion:** These results show that, in a busy diabetic clinic with a diverse ethnic mix, more attention needs to be placed on glycaemic control, both for patients with and without neuropathy. Although all risk factors need to

be treated aggressively, education needs to target patients with neuropathy from ethnic minority groups and especially women of Turkish origin.

## 1018

**Thalamic sensory neuronal dysfunction in distal symmetrical polyneuropathy**

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**Background and aims:** Distal symmetrical polyneuropathy (DSP) has hitherto been considered a disease of the peripheral nerves and little is know on the involvement of the brain. An understanding of the full extent of nervous system involvement in DSP is crucial for the development of rational treatment strategies.
**Materials and methods:** 16 Type 1, male, right handed diabetic patients [8 without DSP (**No-DSP**; Dyck's stage N0) and 8 with DSP (**Est-DSP**; Dyck's stage N1b/2) ] along with 8 healthy male volunteers (**HV**) underwent proton magnetic resonance spectroscopy (H+MRS) of the right posterior lateral nucleus of the thalamus performed at 1.5T (Eclipse, Philips Medical Systems). Proton spectra were obtained from a single voxel (2x2x2 $cm^3$) using short (STEAM: TE = 20 ms, TR = 300 ms) and long (PRESS: TE = 135 ms, TR = 1600 ms) echo-time (TE) techniques. Long TE results are expressed as ratios under the three prominent resonances: Choline (CHO), Creatine (Cr) and N-acetyl (NA) groups. Short TE results are expressed as the areas under the NA, Cho, Cr and *myo*-inositol (mI) resonances relative, to that of unsuppressed water. All volunteers were matched for age and duration of diabetes.
**Results:** In long TE, mean NA:CHO ratio was significantly lower in **Est-DSP** compared to **No-DSP** and **HV** (**Est-DSP** vs **No-DSP**, p=0.036; **Est-DSP** vs **HV**, p=0.015). There was no significant NA differences between **No-DSP** and **HV** (p=0.596). No normalised metabolite short TE differences were seen between the groups (p>0.05).
**Conclusion:** The posterior lateral thalamic subnucleus was chosen because ascending sensory pathways terminate within this nucleus before projections are sent to somatosensory cortical centres. The first H+MRS sequence, which acquired data at short, TE (20 ms) provides information regarding metabolite densities thereby reflecting metabolite concentrations. The second, at long TE (135 ms), yields information about relaxation rates of the neurochemical markers as well as their concentrations. Hence a reduction in NA resonance seen in long TE implies a change in neuronal physiology or function of the ascending sensory nerves in DSP. The short TE results suggest that this is not due to a lower mean concentration in the **Est-DSP** group, which may in turn indicate that the neuronal damage is reversible. There is thus clear evidence for thalamic neuronal dysfunction in **DSP** but further studies are required to determine at what stage during the course of the disease these abnormalities occur.
*Supported by: Diabetes UK*

## 1019

**Influence of vagosympathetic activity on artery rigidity in normotensive and hypertensive Type 2 diabetic patients**

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**Background and aims:** We have previously shown that a high vagal activity may protect against hypertension, and suggested that sympathetic activity may contribute to artery rigidity in type 2 diabetic patients. The aim was here to examine the relationship between cardiovascular vagosympathetic activity and the rigidity of small and large arteries in normotensive hypertensive type 2 diabetic patients.
**Materials and methods:** We included 37 normotensives and 52 age-paired and mildly hypertensives. HbA1 c and serum lipid parameters did not differ significantly between the two groups. The rigidity of large arteries was evaluated by humeral pulse pressure and aortic pulse wave velocity (PWV) (Complior system) and the rigidity of small arteries by pulse pressure of finger arteries (Anapres system). Vagosympathetic activity was evaluated by analysing heart rate (HR) and blood pressure (BP) variations during 6 minutes recordings in the recumbent and the standing position, (Finapres system).
**Results:** Humeral pulse pressure and aortic PWV did not correlate with HbA1 c nor lipids parameters. They were significantly higher in the hypertensive group ($p < 0.0001$ and 0.0003 respectively). Finger artery pulse pressure was also higher ($p < 0.05$). In the hypertensive group, vagal activity (evaluated by the high frequency peak of HR variations) was significantly lower and there was a trend to a higher sympathetic activity (evaluated by the low frequency peak of systolic blood pressure variations).
Humeral pulse pressure and aortic PWV correlated significantly with age ($p < 0.001$ and 0.0004, respectively). In none of the two groups, neither humeral pulse pressure nor PWV correlated with vagosympathetic activity. In the hypertensive group finger pulse pressure correlated with sympathetic activity ($p = 0.03$) and negatively with vagal activity ($p = 0.003$) after age adjustment.
**Conclusion:** In type 2 diabetic patients the rigidity of small and large arteries is higher in hypertensives than in normotensives, the rigidity of large arteries depends mainly on age whereas the rigidity of small arteries depends mainly on vagosympathetic activity.

## 1020

**Hemodynamic and vagosympathetic changes after oral glucose and a mixed meal: influence of Type 2 diabetes and ageing**

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**Background and aims:** Vagosympathetic activity plays a major role in the hemodynamic response to a meal. The postprandial hypotension observed essentially in aged patients with type 2 diabetes (T2D) may result from dysautonomia. The aim of the present study was to investigate the influence of T2D and ageing on the hemodynamic and vagosympathetic responses to glucose and mixed meal intake.
**Materials and methods:** Thirty-four subjects aged < 50 years: 17 T2D (YT2D) and 17 controls (YC) matched for BMI, and 22 subjects > 70 years: 11 T2D (AT2D) and 11 controls (AC) matched for BMI were included. None of them were hypertensive nor had any sign of dysautonomia. Systolic (SBP) and diastolic (DBP) blood pressures and heart rate (HR) were recorded during 120 min after 75 g glucose intake using a Finapres device or during 120 min after meal intake using a Dynamap device.
**Results:** After glucose intake, an initial increase followed by a significant secondary decrease in SBP was observed in AT2D and AC, without significant concomitant change in HR. By contrast, in YT2D and YC, blood pressure remained unchanged and HR significantly increased 90 min after glucose intake. The ratio low/high frequency peaks (LF/HF) of HR variations, an index of vagosympathetic balance, increased 60 min after glucose intake only in YT2D and YC, suggesting a sympathetic activation. Changes in plasma catecholamines concentration did not differ in the four groups. After meal intake, similar patterns of changes in blood pressure and HR were observed in the four groups.
**Conclusion:** These results indicate that 1) hemodynamic response to glucose and not mixed meal is impaired in elderly subject, whatever diabetic or not; 2) the late reduction in blood pressure may explain some nonhypoglycemic postprandial dizziness observed in AT2D, and the role of high glycaemic index foods may be hypothesized; 3) the lack of a parallel increase in HR may result from baroreflex impairment with an insufficient sympathetic activation.

## 1021

**A new treatment for painful diabetic neuropathy: the Frequency Modulated Neural Stimulation (FREMS)**

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**Background and aims:** Painful peripheral neuropathy causes considerable disability and impairs the quality of life of many diabetic patients. Moreover, its treatment is often difficult and unsatisfactory. Aim of our study was to evaluate the efficacy of Frequency Modulated Neural Stimulation (FREMS) for the management of painful neuropathy in patients with diabetes. FREMS is a novel transcutaneous electrotherapy characterized by sequences of high voltage and low pulse duration electrical stimuli which vary in both frequency and duration. Preliminary experimental data suggest that a possible mechanisn of action of FREMS is the local release of angiogenic factors which possibly increase the nerve blood flow.
**Materials and methods:** thirtyfour patients with type 1 (n=7) or type 2 (n=27) diabetes with painful neuropathy and decreased nerve conduction velocities (≤40 m/sec) were enrolled in this randomised, double-blind,

crossover FREMS vs placebo (undistinguishable nonsense electrostimulation) study. Each patient received in random sequence two series of 10 treatments of either FREMS or placebo at intervals of at least 24 hr from each other, lasting no more than 3 weeks per series. At the beginning (T0) and at the end (T1) of each treatment period, the intensity of pain assessed by visual analog scale (VAS), vibration perception threshold (VPT) measured at both big toes by Biothesiometer and motor (MNCV) and sensory (SNCV) nerve conduction velocities were obtained from all the patients.
**Results:** compared to placebo FREMS reduced both diurnal (FREMS: T0= 41.4 ± 4.9, T1= 30.5 ± 3.8, p=0.002; placebo: T0= 35.4 ± 4.0, T1= 35.1 ± 3.7, p=NS) and nocturnal (FREMS: T0= 41.0 ± 5.2, T1= 29.6 ± 4.0, p=0.01; placebo: T0= 37.5 ± 4.0, T1= 32.9 ± 4.0, p=NS) VAS scores. VPT was also improved by FREMS (FREMS: T0= 33.7 ± 1.7 V, T1= 31.1 ± 1.9 V, p<0.0001; placebo: T0= 32.6 ± 1.7 V, T1= 32.4 ± 1.7 V, p=NS) as well as MNCV (FREMS: T0= 35.6 ± 1.1 m/sec, T1= 40.1 ± 1.5 m/sec, p=0.0008; placebo: T0= 37.0 ± 1.0 m/sec, T1= 37.2 ± 1.2 m/sec, p=NS). On the contrary, SNCV was not significantly changed by FREMS although a trend towards improvement was seen. At 4-month follow-up visit, significant benefit persisted for pain score only (p<0.05 vs baseline).
**Conclusion:** these findings indicate a beneficial effect of FREMS on neuropathic pain and objective measures of nerve function in diabetic patients with painful peripheral neuropathy.

# PS 99
# Experimental neuropathy

## 1022

**Systematic morphological evaluation of peripheral sensory and motor nerve fibers and endoneurial microvasculature in rats with insulinoma: evidence for sciatic nerve endoneurial infarction**
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**Background and aims:** Hyperinsulinemia and hypoglycemia can alter peripheral nerve function and structure as well as nerve blood flow. In patients with insulinoma, neurological signs and symptoms suggesting peripheral sensory and motor nerve involvement may be a major clinical feature. However, none of the previous study conducted a systematic quantitative analysis of the morphological alterations in peripheral sensory and motor nerve fibers as well as in endoneurial microvasculature in this syndrome. We, therefore, attempted to characterize neuropathic changes associated with chronic hyperinsulinemia and hypoglycemia secondary to insulinoma in rats.
**Materials and methods:** We undertook structural, morphometric, and teased fiber analysis of myelinated fibers and examined endoneurial microvascular abnormalities at different levels of the peripheral pure sensory, pure motor, and mixed sensory motor nerves of the hind limbs in longstanding insulinoma-carrying rats (n=12, I-rats). Age-matched normal rats (n=6) served as control.
**Results:** Over the 15-month observation period, two of I-rats developed paresis of the hind limbs when their blood glucose level fell below 1.7 mmol/l. These animals showed a massive myelinated fiber loss and multiple endoneurial microvascular occlusions at the sciatic nerve level. In the rest of non-paretic I-rats, there was a loss and degeneration of large myelinated fibers in the ventral root and peroneal nerve, while small myelinated fibers were increased without showing degenerative changes in the sural nerve. There were conspicuous endoneurial microangiopathic changes including endothelial swelling, narrowed lumina, and vascular wall thickening in the sciatic and peroneal nerves, while endoneurial microvascular density was increased in the sciatic and sural nerves. Ventral horn motor neurons in the lumbar spinal cord and sensory neurons in the lumbar DRG appeared normal in these animals including those with paresis.
**Conclusion:** The present study provides the first evidence for the occurrence of the sciatic nerve endoneurial infarctions in I-rats under extreme condition. We also suggest that the observed increase in the sensory nerve endoneurial microvascular density in non-paretic I-rats may be a compensatory response to endoneurial ischemia/hypoxia associated with insulinoma, thereby contributing to the preserved morphology of the sensory myelinated fibers in these animals. In conjunction with previous data, it is likely that hypoglycemia is harmful to large motor myelinated fibers, while hyperinsulinemia is associated with increased small sensory myelinated fibers and sensory nerve endoneurial microvascular density.

## 1023

**Role for nitrosative stress in diabetic neuropathy: evidence from studies with a peroxynitrite decomposition catalyst**
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**Background and aims:** The potent oxidant peroxynitrite, a product of superoxide reaction with nitric oxide, is known to play a major role in poly(ADP-ribose) polymerase (PARP) activation and other detrimental consequences in numerous pathological conditions associated with oxidative stress. The evidence of the presence of nitrosative stress i.e. enhanced peroxynitrite formation in both experimental and clinical diabetic neuropathy (DN) is emerging; however, its pathogenetic role in neuropathic changes has not been elucidated. This study was designed to evaluate the role for nitrosative stress in DN in two models of Type 1 diabetes, streptozotocin (STZ)-diabetic mice and diabetic NOD mice.
**Materials and methods:** Control (C) and STZ-diabetic (D) mice were treated with/without the potent peroxynitrite decomposition catalyst FP15 (Inotek Pharmaceuticals Corp., 5 $mg.kg^{-1}.d^{-1}$) for 1 wk after initial 8 wks without treatment. Diabetic NOD mice were treated with either 1 or

3 mg.kg$^{-1}$.d$^{-1}$ FP15 for 1 wk. The agent was administered in the drinking water. Sciatic nerve nitrotyrosine and poly(ADP-ribose) accumulation was evaluated by immunohistochemistry. Sciatic nerve concentrations of glucose, sorbitol pathway intermediates and variables of energy state i.e. phosphocreatine and creatine were measured spectrofluorometrically by enzymatic procedures.
**Results:** Sciatic nerve nitrotyrosine (a marker of peroxynitrite-induced injury) and poly(ADP-ribose) accumulation were present in D, and absent in C and D+FP15. FP15 essentially corrected sciatic motor and hind-limb digital sensory nerve conduction deficits as well as sciatic nerve energy state (assessed from phosphocreatine/creatine ratio) in D, without affecting those variables in C. Nerve glucose and sorbitol pathway intermediate concentrations were similarly elevated in D and D+F vs C. In diabetic NOD mice, a 7-d treatment with either 1 or 3 mg kg-1 d-1 FP15 reversed increased tail-flick latency (a sign of reduced pain sensitivity); the effect of the higher dose was significant as early as 3 d after beginning of the treatment.
**Conclusion:** In conclusion, nitrosative stress plays a major role in DN in, at least, Type 1 diabetes. The study provides the rationale for development of agents counteracting peroxynitrite formation and promoting peroxynitrite decomposition, and their evaluation in DN.
*Supported by: NIH/NIDDK (CS, IGO), ADA (IGO)*

## 1024

**Orexin B-induced increase in spontaneous firing in hypothalamic paraventricular nucleus neurons was suppressed in streptozotocin-diabetic rats**

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**Background and aims:** Orexin-B plays a role in the central mechanisms that regulate feeding and sleep/arousal. The most intense hypothalamic immunostaining against orexin-2 receptors is obtained in the paraventricular nucleus (PVN) of rat hypothalamus. We have previously reported that orexins lower the blood glucose levels (BGL) especially in the fasting state in streptozotocin (STZ)-diabetic mice. The present study is aimed to elucidate whether the electrophysiological profiles of orexin B actions in PVN are changed in diabetic state.
**Materials and methods:** Wistar male rats (3 weeks old) were singly injected with STZ (60 mg/kg, i.v.) under ether anesthesia. The rats with STZ-diabetes (BGL: 477 ± 24 mg/dl) were used 4–5 weeks after the injection. BGL of age-matched normal rats were 104 ± 3 mg/dl. The BGL were measured with the glucose-oxidase methods (ANTSENSE II). The brain slices of rats in fasting state were isolated under ether anesthesia, and immersed in artificial cerebrospinal fluid (NaCl 124, KCl 5.0, $CaCl_2$ 2.4, $MgSO_4$ 1.3, $KH_2PO_4$, $NaHCO_3$ 26, and glucose 10 mM). Spontaneous firings in PVN magnocellular neurons in brain slices were extracellularly recorded, counted, and analyzed by unpaired *t*-test.
**Results:** 1. Basal spontaneous firing frequencies in PVN magnocellular neurons of brain slices were not different between normal rats (337 ± 99 sec$^{-1}$, n = 18) and diabetic rats (411 ± 127 sec$^{-1}$, n = 12). 2. Bath-application of orexin B (0.01, 0.1 and 1 μM; for 3 min) increased spontaneous firing frequency in a concentration-dependent manner in the same area: the frequencies were 0.25 ± 0.04 sec$^{-1}$ (n = 4), 1.51 ± 0.34 sec$^{-1}$ (n = 10), and 2.06 ± 0.62 sec$^{-1}$(n = 4), respectively. In the diabetic state, the responses to orexin B (0.1 μM) were significantly suppressed to 0.66 ± 0.11 sec$^{-1}$ (n = 6, P<0.05).
**Conclusion:** In the hypothalamus, orexin neurons provide innervation to the arcuate nucleus and PVN. The PVN is an important region for the integration of neuroendocrine and autonomic functions. In diabetic state, the c-fos expression levels were abnormally elevated in the PVN magnocellular neurons, mainly composed of oxytocin- and vasopressin-producing cells. Since the orexin B-induced firings in PVN magnocellular neurons were suppressed in diabetic state, we suggest that the regulation by orexin B of these neuroendocrine systems is perturbed in diabetic brain. Thus, the central orexin actions involving feeding and energy homeostasis may be unusual in diabetic state.

## 1025

**Effects of benfotiamine on vascular endothelium and nerve function and perfusion in diabetic rats**

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**Background and aims:** Benfotiamine is a vitamin B1 analogue that has previously been shown to have beneficial effects on retinal and renal complications in diabetic rats. The likely mechanism of action is to reduce the accumulation of sugar phosphates; hence decreasing advanced glycation end product formation. The aim was to examine whether benfotiamine treatment could also improve small and large diameter nerve fibre and vascular endothelium function in diabetic rats.
**Materials and methods:** Diabetes was induced by streptozotocin; duration was 24 or 8 weeks. Benfotiamine treatment (75 mg/kg p.o.) was given for the last 2 weeks. Measurements were made on nerve electrophysiology (sensory and motor conduction velocity), and nerve perfusion by hydrogen clearance microelectrode polarography. Renal artery endothelial function and gastric fundus non-adrenergic non-cholinergic (NANC) innervation were studied in vitro. Data are mean ± SEM.
**Results:** After 24 weeks of diabetes, large fibre measures of sciatic motor and saphenous nerve sensory conduction velocities were reduced by 19.3 ± 1.2% and 19.9 ± 1.4% respectively in diabetic rats. Two weeks of benfotiamine treatment corrected the motor deficit by 74.6 ± 7.9% (p<0.001) and the sensory deficit by 91.7 ± 5.2% (p<0.001). Sciatic nerve nutritive endoneurial blood flow was 48.6 ± 3.2% reduced (p<0.001) by diabetes. This deficit was partially reversed (62.6 ± 7.8%; p<0.001) by 2 weeks of benfotiamine treatment. The small fibre NANC innervation of gastric fundus strips was studied after 8 weeks of diabetes. After serotonin precontraction, in the presence of guanethidine and atropine, electrical field stimulation caused frequency-dependent relaxations that were depressed by diabetes, the maximal relaxation deficit being 49.3 ± 5.2% (p<0.001). This defect was 55.5 ± 12.9% corrected (p<0.001) by 2 weeks of benfotiamine treatment. Experiments in the presence of nitric oxide (NO) synthase blockade showed that the benefits of benfotiamine were on the nitrergic rather than the peptidergic NANC relaxation component. Phenylephrine-precontracted renal artery rings showed a 41.9 ± 6.9% reduction (p<0.001) in endothelium-dependent relaxation to acetylcholine after 8 weeks of diabetes, which was 62.1 ± 18.3% corrected (p<0.05) by 2 weeks of benfotiamine treatment. This response is mediated by both NO and endothelium-derived hyperpolarizing factor (EDHF). When the EDHF component was isolated by NO synthase blockade, an 86.4 ± 7.8% diabetic deficit (p<0.001) was revealed which was unaffected by benfotiamine treatment. Neither diabetes nor benfotiamine treatment affected endothelium-independent relaxation to the NO donor, sodium nitroprusside.
**Conclusion:** Benfotiamine treatment reversed some of the neural and vascular deficits in diabetic rats. There were marked benefits for NO-mediated processes. Thus benfotiamine may have a potential therapeutic role in diabetic neuropathy and vasculopathy, which requires further study.

## 1026

**Aldose reductase inhibitor fidarestat counteracts nitrosative stress and poly(ADP-ribose)polymerase activation in experimental diabetic neuropathy**

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**Background and aims:** Studies with aldose reductase inhibitors (ARIs), AR-overexpressing and AR-knockout models indicate that AR is a major contributing factor to oxidative stress in tissue-sites for diabetic complications. Recently, one group reported that oxidative stress and poly (ADP-ribose) polymerase (PARP) activation is a cause of diabetes-induced sorbitol pathway hyperactivity. This study evaluated the effects of AR inhibition on nitrosative stress and PARP activation in experimental diabetic neuropathy (EDN). Nitrosative stress i.e. increased formation of peroxynitrite, the product of superoxide reaction with nitric oxide, is known to be largely responsible for poly(ADP-ribosyl)ation in numerous pathological conditions associated with oxidative injury.
**Materials and methods:** Control (C) and steptozotocin-diabetic (D) rats were treated with/without the ARI fidarestat (F, 16 mg.kg$^{-1}$.d$^{1}$) for 6 weeks starting from induction of diabetes. Nitrotyrosine (NT, a marker of peroxynitrite-induced injury) and poly (ADP-ribose) accumulation in sciatic

nerve, and superoxide and NT abundance in isolated epineurial vessels were assessed by immunohistochemistry. Sciatic nerve glucose, sorbitol pathway intermediate, GSH and ascorbate concentrations were measured by spectrofluorometric procedures, and fidarestat levels by LC/MS/MS. Intracellular oxidative stress in endothelial cells cultured in 5 mM glucose or 30 mM glucose with/without 1 μM F was evaluated using the CM-$H_2$DCFDA fluorescent probe and flow cytometry.
**Results:** Sorbitol pathway intermediate, but not glucose, accumulation was completely prevented in D+F. F counteracted increased superoxide formation in epineurial vessels, and *in vitro* studies confirmed the endothelial origin of this phenomenon. F prevented NT accumulation in *vasa nervorum,* and both NT and poly(ADP-ribose) accumulation in sciatic nerve, probably due to preservation of two major non-enzymatic antioxidants, GSH and ascorbate. At the concentrations quantified in sciatic nerves in D+F, F did not exhibit direct antioxidant properties, and did not cause direct inhibition of PARP activity in cell-free system, containing PARP and $NAD^+$.
**Conclusion:** AR inhibition counteracts nitrosative stress and PARP activation in EDN. These findings reveal new beneficial properties of F thus further justifying the ongoing clinical trials of this specific, potent and low-toxic ARI.
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## 1027

**Rosuvastatin exerts beneficial effects on microcirculatory and electrophysiological changes in diabetic rats, independently of its lipid lowering effect**
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**Background and aims:** Statins may exert various beneficial effects on microcirculation independently of its lipid lowering effect. We have previously shown that cerivastatin prevents microcirculatory disorders and to a lower extent nerve dysfunction in diabetic rats. The aim was to test the effect of rosuvastatin and its mechanism, on microcirculatory and neurophysiological changes induced by diabetes.
**Materials and methods:** 45 male Wistar rats with diabetes induced by STZ at 5 days were randomised in 3 groups : rosuvastatin (R), mevalonate and rosuvastatin (MR), untreated group (U). At T0, at a mean age of 3 months, (R) received 20 mg/kg/day of rosuvastatin and (MR) received the same dosage of rosuvastatin and 20 mg/kg/day of mevalonate. Motor and sensory nerve conduction were analysed at T0 and when the rats were aged of 8 months (T2). At T0 and T2, capillary filtration of albumin and lymphatic function were also investigated by a noninvasive method using $^{99m}$-Tc-labelled albumin. Data were compared with a control group (15 normal Wistar rats).
**Results:** At T0, no differences were found neither for nerve conduction parameters nor for microcirculatory parameters between the 4 groups. At T2, motor nerve velocity and CMAP amplitude were decreased in the 3 diabetic groups, sensory nerve conduction velocity values in groups R and MR were intermediate between values for group U and the control group. There was no significant difference between the 3 diabetic groups regarding SNAP amplitude, sensory area and sensory potential duration. The latency of peak 1 of sensory nerve potential was significantly longer in group U than in controls ($p < 0.02$) and groups R and MR ($p = 0.02$), without difference between the control group and groups R and MR. Regarding the isotopic test, interstitial albumin retention and lymphatic uptake of interstitial albumin were significantly impaired in group U, and these changes were prevented in groups R and MR.
**Conclusion:** 1) Rosuvastatin exerts a preventive effect on diabetic sensory nerve function deterioration as shown mainly by an improvement in large fiber sensory conduction. 2) Since this beneficial effect is not suppressed by mevalonate, it is consistent with a pleiotropic effect. 3) The preventive effect on capillary filtration and lymphatic dysfunction might be involved in nerve function improvement through a reduction in endoneurium edema.

# PS 100
# Autonomic neuropathy

## 1028

**The effect of epalrestat in cardiac sympathetic nerve dysfunction with Type 2 diabetes**
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**Background and aims:** Diabetic patients have the potential for complications of microcirculatory failure and autonomic nervous system dysfunction in the heart, and therefore they often experience heart failure and sudden death. Recently, the stage of this abnormal condition has been evaluated using cardiac nuclear medical approaches, such as adenosine triphosphate (ATP) stress thallium-201 myocardial scintigraphy and I-123 metaiodobenzylguanidine (MIBG,analogue of norepinephrine) myocardial sympathetic nerve function scintigraphy. We have previously investigated the relationship between these approaches and blood sugar control in diabetic patients. In this study, we investigated whether epalrestat, an aldose reductase inhibitor, improved autonomic nervous system dysfunction, using MIBG myocardial sympathetic nerve function scintigraphy.
**Materials and methods:** The subjects were 24 patients with type II diabetes who had no abnormal findings in ECG and cardiac echo study(male:10,female:14, mean age: 64.9 ±yrs.). All patients first underwent MIBG myocardial sympathetic nerve function scintigraphy. MIBG myocardial sympathetic nerve function scintigraphy was performed 30 min.after injection of MIBG and at 4-hr delayed interval and washout-rate (MIBG-WR) were calculated as well as the heart to mediastinum ratio (H/M) at delayed planar images .The subjects were randomly allocated into two groups: twelve patients who were given epalrestat, and twelve who did not receive epalrestat. All subjects then underwent MIBG myocardial sympathetic nerve function scintigraphy again one year later. The blood sorbitol concentration was determined before and one year after epalrestat administration.
**Results:** All except two subjects showed abnormally high values of MIBG-WR. Subjects of the epalrestat-treated group were classified into two subgroups by their MIBG-WR value before epalrestat administration, i.e., the high MIBG-WR group (45 or more; Group A) and the low MIBG-WR group (less than 40; Group B). One year after epalrestat administration, subjects in Group A showed aggravation (before epalrestat administration: $46.2 \pm 1.1$,one year after: $49.7 \pm 1.9$ $p < 0.01$), while subjects of Group B showed improvement or no change (before epalrestat administration: $32.9 \pm 1.0$,one year after:$30.9 \pm 1.2$). Subjects who did not receive epalrestat (Group C) showed a level of aggravation similar to that of Group A. No significant changes in H/M were found in any group. The subjects of both Group A and B showed improvement in blood sorbitol, but without any significant difference between the two groups.
**Conclusion:** Epalrestat administration had no effect on cardiac sympathetic dysfunction,which was identified from the MIBG-WR value, in type II diabetes patients with severe dysfunction. However, epalrestat may inhibit aggravation of dysfunction and may improve the condition of patients with mild dysfunction.

## 1029

**Influence of blood glucose control on the progression of cardiac autonomic neuropathy in Type 1 diabetes**
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**Background and aims:** Cardiac autonomic neuropathy (CAN) is a frequent complication of diabetes mellitus. The present work aimed at analysing the influence of blood glucose control on the progression of indices of CAN in patients with type 1 diabetes mellitus.
**Materials and methods:** 48 (28 women and 20 men) patients with type 1 diabetes (mean ± SD : age : 41 ± 12 years; diabetes duration : 19 ± 10 years with a range from 3 to 38 years) were studied twice at a time interval of 43 ± 17 months (range: 16–91 months). Patients taking drugs interfering with cardiovascular regulation were excluded. CAN was assessed by calculating the baroreflex gain during a squatting test (1 min standing – 1 min squatting – 1 min standing) while continuous monitoring of heart rate and arterial blood pressure with a Finapres$^R$ device. The baroreflex gain was calculated by plotting R-R cardiac intervals according to mean arterial blood pressure values during the transition active phase from squatting to standing. Overall blood glucose control was estimated by the mean value of several $HbA_{1c}$ measurements between the two tests, and subjects were

separated into two subgroups: $HbA_{1c}$ levels ≤ 8 % in 21 patients (7.42 ± 0.60 %) *versus* > 8 % in 27 patients (9.44 ± 1.04 %).
**Results:** The baroreflex gain tended to be negatively related to the duration of diabetes at the initial evaluation ($r = -0.174$; $n = 48$; $p < 0.10$). This index of CAN decreased from 4.00 ± 3.41 to 2.53 ± 1.46 msec/mm Hg ($p = 0.008$) during the 43-month period separating the two orthostatic tests. The reduction in baroreflex gain was significant in patients with bad glucose control (from 3.89 to 2.13 msec/mm Hg, $p = 0.02$), but not in patients with acceptable control (from 4.16 to 3.05 msec/mm Hg, NS). While no significant differences were observed between the two groups at the initial evaluation, baroreflex gain became significantly lower in the poorly controlled group than in the better controlled group at the second evaluation ($p < 0.05$). A significant negative correlation was found between changes in baroreflex gain from test 1 to test 2 and mean $HbA_{1c}$ levels ($r = -0.304$; $n = 48$; $p < 0.01$), and between baroreflex gain at the second test and averaged $HbA_{1c}$ during the period between the two tests ($r = -0.409$; $n = 48$; $p < 0.001$). Interestingly, pulse pressure (arterial systolic minus diastolic blood pressure) significantly increased from test 1 to test 2 in patients with poor metabolic control (from 47 to 58 mm Hg; $p < 0.001$), but not in patients with better metabolic control (from 50 to 57 mm Hg; NS).
**Conclusion:** In patients with long-standing (almost 20 years) type 1 diabetes, a period of 3–4 years with poor glucose control is sufficient to significantly decrease baroreflex gain, an index of cardiac autonomic neuropathy, and to increase pulse pressure, an index of arterial stiffness, two independent risk markers in patients with diabetes mellitus. These observations support the search of better metabolic control in patients with type 1 diabetes, with a minimum objective of $HbA_{1c}$ levels below 7%.

## 1030

**Effects of acute hyperglycemia on QT interval, and sympatho-vagal balance in Type 2 diabetic patients with and without autonomic neuropathy**

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**Background and aims:** Type 2 diabetic patients display increased cardiovascular mortality and sudden deaths. Abnormalities in QT duration, possibly related to diabetic autonomic neuropathy (AN), may play a role. Moreover, AN is associated with an alteration of the circadian rhythm of the sympatho-vagal balance, with a relative increase of the sympathetic (LF) over the parasympathetic (HF) component during the night. It has been shown that acute hyperglycemia affects sympathetic activity and QT duration in normal individuals.
The aim of the present study was to evaluate the effects of acute hyperglycemia on QT duration and sympathetic activity both during the 24 h period and in response to acute hyperglycaemia in type 2 diabetic patients, with and without AN.
**Materials and methods:** we studied 30 type 2 diabetic subjects (D), 11 with (N+) and 19 without (N-) AN, and 13 non diabetic control subjects (C). After a 15 day wash-out period, all subjects underwent an ECG Holter and blood pressure monitoring during the 24 h period and during a 2 h hyperglycaemic clamp. QT duration and sympatho-vagal balance (LF/HF) were evaluated for the 24 h period and in response to hyperglycemia
**Results:** during the 24 h period, SBP during the night was significantly increased in D vs. C (123.45 ± 10.85 vs. 114.23 ± 7.94 mmHg; p= 0.009), and ΔSBP was significantly reduced in D vs. C (9.51 ± 4.86 vs. 14.23 ± 6.44, p= 0.01). ΔLF/HF was also significantly reduced in D vs C (6,64 ± 38,6 vs. 37,55 ± 60; p= 0.05). QTc during the night was lengthened in D vs. C (411 ± 18,94 vs. 397,23 ± 20.16 mms; p <0.05) and in N+ vs. C (419.20 ± 26.18 vs. 397.23 ± 20.16 mms; p= 0.04). ΔQTc was significantly reduced in D vs. C (0.33 ± 2.47 vs. 3.39 ± 2.32; p= 0.001) and in N+ vs. C (0.29 ± 2.27 vs. 3.39 ± 2.32; p= 0.006). Acute hyperglycemia significantly increased SBP only in C (132.86 ± 14.56 vs. 127.55 ± 12.53 mmHg; p= 0.03), but not in D (135.78 ± 15.03 vs. 135.51 ± 15.07; p= ns). Moreover, at the end of the clamp study, LF/HF was significantly increased in C and N- (3.64 ± 2.34 vs. 3.00 ± 2.24; p= 0.03), but not in N+ (2.67 ± 0.95 vs. 2.58+2.09; p= ns). A correlation between 2 h LF/HF and 2 h SBP ($r^2 = 0.7$), was observed only in C. QTc, basally increased in N+, was significantly increased by acute hyperglycemia only in N- (399.70 ± 14.19 vs. 395.53 ± 13.26; p= 0.01) but not in N+ (422.71 ± 27.13 vs 415.40 ± 25.38; p= ns).
**Conclusion:** These results suggest that the impaired sympatho-vagal balance, observed in diabetic patients with autonomic neuropathy affects the circadian rhythm of blood pressure leading to a reduced nocturnal fall of blood pressure and a lenghtening of the QTc interval, during the night. The lack of effects of acute hyperglycaemia on QT and on the behaviour of sympatho-vagal balance suggests that autonomic neuropathy plays an important role in influencing cardiac parameters in response to hyperglycemia.

## 1031

**Cardiac autonomic function related to HOMA-IR in hypertension with or without associated diabetes mellitus**

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**Background and aims:** It is well known that cardiac autonomic neuropathy (CAN) is a strong predictor of cardiovascular mortality, but also that its development is related to cardiovascular risk factors. It develops not only by well-established diabetes, but also at any stage of abnormal glucose metabolism, and that hypertension itself is a risk factor for CAN. Cardiac autonomic neuropathy has been associated with poor prognosis, however the mechanisms remain a matter of debate. The aim of our study was to determine whether there is any correlation between cardiac autonomic function and insulin resistance in patients with hypertension with and without type 2 diabetes.
**Materials and methods:** The standard Ewing cardiovascular reflex test battery was performed in 81 hypertensiv subjects, 38 with and 43 without type 2 diabetes (mean age: 54,7 ± 12,1/59,7 ± 10,5 years), and in a control group, consisting of 59 healthy adults (mean age 48,6 ± 13,7years). In each group BMI, basal insulinaemia, with conventional insulin assays, fasting glicaemia, resting blood pressure (BP), microalbuminuria (MA), QTc interval (on surface ECG using Bazzet's formula) were assessed. HOMA-IR was calculated with the formula: insulin (microU/ml) × glycaemia (mmol/l)/22,5. CAN was characterised as an abnormality of more than two cardiovascular tests. We compared Ewing test results in the three above-mentioned groups, and between groups defined as insulin resistant and as non-resistant, using as cut-off point the upper quartile HOMA-IR value of the control group (2,9).
**Results:** HOMA-IR was significantly higher in hypertensiv subjects with diabetes, than without diabetes (5,3 vs. 3,9 p<0,05), and in both of them were significantly higher than in control group (2,13 p<0,005). Autonomic neuropathy was more often present in diabetes patients (53% vs. 9%, p<0,01), and in those insulin resistant (62% vs. 38%, p<0,05) The insulin resistant group had also higher heart rate (78 vs. 68, p<0,05), longer QTc (0,39 vs. 0,32, p<0,05), and greater MA (22,6 vs. 12,5, p<0,05). There was a strong correlation between parameters of cardiac reflex tests and MA (r=0.389, p<0,05), and HOMA-IR and MA (r=0,365, p<0,05) There was no significant difference between the studied groups in BMI, BP.
**Conclusion:** Insulin resistance could be a basic risk factor for the development of autonomic neuropathy and abnormal autonomic cardiovascular function.

## 1032

**Determinants and diagnostic usefulness of spontaneous baroreflex sensitivity (BRS) in Type 1 diabetic patients**

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**Background and aims:** To investigate clinical determinants and diagnostic accuracy of spontaneous BRS in type 1 diabetes.
**Materials and methods:** In 34 type 1 diabetic patients (age 34 ± 11, duration 15 ± 10 years, BMI 25 ± 4 Kg/m$^2$, HbA1 c 7.8 ± 1.6%, 15 men), we performed 4 cardiovascular tests, 24 h BP monitoring, and short-term (5 min supine and 5 min standing) simultaneous autoregressive spectral analysis of RR interval and BP recordings to measure the low- (LF) and high-frequency (HF) spectral components and the spontaneous BRS using the alpha index, i. e. the squared ratio of the spectral powers of RR interval and systolic BP, as: $[(LF_{RR}/LF_{SBP})^{0.5}+(HF_{RR}/HF_{SBP})^{0.5}]$:2. 40 healthy subjects (C) (age 37 ± 11 years, BMI 24 ± 4 Kg/m$^2$, 19 men) were used as controls for BRS.
**Results:** According to cardiovascular tests, 21 patients had autonomic neuropathy (AN+) (at least 1 abnormal test) and 13 did not (AN-). Supine alpha index was significantly lower in AN+ than in both C (5.8 ± 4.7 Vs 14.6 ± 8 ms/mmHg, p<0.001) and AN- (5.8 ± 4.7 Vs 17.2 ± 13.7 ms/mmHg, p<0.01). Standing alpha index was significantly lower in AN+ than in AN- (2.1 ± 1.6 Vs 8.5 ± 12.7 ms/mmHg, p<0.01). Alpha index was not significantly different between AN- and C. Assuming a value of 3 and 2 ms/mmHg (the 5$^{th}$ percentile of control observations) as the lower limit of supine and standing alpha index, the sensitivity and specificity of BRS in identifying AN+ patients were 48% and 83%, respectively. Low BRS was associated with microalbuminuria ($Chi^2=5.3$, p<0.05), hypertension ($Chi^2=10.7$,

p<0.01), and sedentary lifestyle ($Chi^2$=4.2, p<0.05). Patients with low BRS compared to those with normal BRS, showed higher autonomic score (4.9±2.5 Vs 1.9±1.9, p<0.001), higher 24h BP (24h DBP: 79±9 Vs 71±6mmHg, p<0.01), and lower day-night difference (Δ) in BP (Δ DBP: 7±6 Vs 18±8%, p<0.001). In univariate analysis supine alpha index was related to age (r=−0.40, p<0.05), autonomic score (r=−0.54, p<0.01), 24h BP (r=−0.39, p<0.05), Δ BP (r=0.54, p<0.01), in addition to $LF_{RR}$ and $HF_{RR}$ spectral powers (r=0.79, p<0.001). In a stepwise regression analysis including age, autonomic score, albumin excretion rate, physical activity, and 24h DBP, BRS was still related only to the autonomic score and age.
**Conclusion:** Although BRS derived from spectral analysis of RR and BP is closely linked to the other autonomic indexes, this study does not confirm its value as a very sensitive marker of autonomic dysfunction in type 1 diabetes.

## 1033

**Serum concentrations of adiponectin are associated with sympatho-vagal balance evaluated by power spectral analysis of heart rate variations in patients with Type 2 diabetes**
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**Background and aims:** To investigate whether cardiac autonomic activities or sympathovagal balance as estimated by power spectral analysis of HRV was associated with serum concentrations of adiponectin in type 2 diabetic patients.
**Materials and methods:** We studied 105 type 2 diabetic patients (51 female and 54 male). Serum concentrations of adiponectin were measured by a sandwich enzyme-linked immunosorbent assay. HRV was automatically determined every 5 minutes during 24 h with a Holter electrocardiogram 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:** We found no significant correlation between serum adiponectin and LF or HF power in diabetic patients. Serum adiponectin was negatively correlated with 24-h LF/HF ratio (r= −0.343, P=0.0009) and creatinine clearance (r = −0.411, P<0.0001). Serum adiponectin concentrations were significantly higher in the patients with overt albuminuria than in those with normoalbuminuria or microalbuminuria. Multivariate analysis controlling for gender, age, BMI, glycemic control, lipids profile and renal function showed a strong independent negative association of serum adiponectin with the 24-h LF / HF ratio (partial coefficient = −0.326, P = 0.027).
**Conclusion:** Serum concentrations of adiponectin were associated with a shift of the sympathovagal balance toward a sympathetic activation, suggesting that sympathetic predominance is an independent determinant of serum adiponectin in type 2 diabetes. Serum adiponectin also was independently influenced by impaired renal function.

# PS 101
# Erectile dysfunction

## 1034

**Comprehensive assessment of clinical, socioeconomic (SE) and lifestyle parameters associated with erectile dysfunction (ED) among diabetic men**
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**Background and Aims:** Diabetes and non-diabetes related factors may underlie ED in diabetic men. We assessed the association between ED and a comprehensive set of clinical, SE and lifestyle parameters among male diabetic patients.
**Materials and Methods:** Adult men (age >18 years), treated in 26 diabetes clinics in Israel were randomly selected. Participants completed a self-reported questionnaire on demographic, SE and lifestyle characteristics, and on erectile function (EF), using the International Index of Erectile Function (IIEF-15). Information on diabetes type, duration, treatment and control, presence of microvascular complications and cardiovascular disease (CVD), medical treatment, blood pressure and lipid levels was obtained from the patients' medical records.
**Results:** Out of 1,510 eligible men, 1,301 (86%) were recruited and 1,040 provided complete information on EF (overall response rate: 69%). Patients mean age (SD) was 57 (12) years and 88% of them had type-2 diabetes. The median diabetes duration was 8 years (range: <1–50). In addition to diabetes, 46% had hypertension (HT) and 53% had dyslipidemia. About half of the patients had microvascular complications in ≥ 1 target organ and 25% had CVD. Normal EF was found in 13.5% of the patients, 20.8% had mild ED, 18.6% had mild to moderate ED, 17.1% had moderate ED and 30.1% had severe ED. The following characteristic were found to be significantly and independently associated with EF, presented as a 5-category ordinal variable, in a multivariate ordinal logistic regression analysis:

| Characteristic | Adjusted OR (95%CI) | P |
|---|---|---|
| Age (5 years increment) | 1.38 (1.29, 1.48) | <0.0001 |
| Diabetes duration (5 years increment) | 1.16 (1.07, 1.26) | 0.0006 |
| Current HbA1 c (1% increment) | 1.10 (1.01, 1.19) | 0.0253 |
| CVD | 1.78 (1.27, 2.48) | 0.0008 |
| Any microvascular disease | 1.43 (1.09, 1.88) | 0.0107 |
| Diuretics | 1.78 (1.09, 2.91) | 0.021 |
| Alcohol drinking (≥ 1 unit/week) | 0.70 (0.51, 0.97) | 0.0296 |
| Physical activity (vs. none): | | |
| -Non-leisure | 0.82 (0.56, 1.21) | 0.002 |
| -Leisure | 0.77 (0.55, 1.08) | |
| -Both | 0.51 (0.36, 0.72) | |

SE parameters, cigarette smoking, diabetes type, dyslipidemia, HT and use of other antihypertensive drugs were not found to be significantly associated with ED in the multivariate model.
**Conclusion:** Among diabetic men, ED is associated with higher age and longer diabetes duration, poor glycemic control, microvascular complications, diuretic treatment and CVD. Physical activity and alcohol intake are protective. These findings may have preventive implications and help physicians to target early screening and treatment at high-risk patients.
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## 1035

**National survey on the prevalence and risk factors of erectile dysfunktion in Japanese men with Type 2 diabetes**

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**Background and aims:** Since we have been able to use the phosphodiesterase 5 inhibitor, sildenafil, erectile dysfunction (ED) has been considered to be a disease which could easily be treated. On the other hand, it has been reported that diabetic men often suffer from ED. Therefore, it is important to evaluate the prevalence and risk factors of ED and the demand for ED treatment in the diabetic population from the viewpoint of what will ensures a patients' quality of life (QOL). In this study, we report the present status of ED in Japanese male diabetics.
**Subjects and methods:** A national survey questionnaire was given to 119 general practice physicians and their outpatients from in Japan. The same questionnaire was also administered to 25 male non-diabetic healthy subjects. The following parameters were investigated: age, ED severity, glycemic control, diabetic microangiopathy (nephropathy, retinopathy, neuropathy), hypertension, hyperlipidemia, cerebro- or cardiovascular disease, therapy for diabetes, subjective symptoms related to QOL or diabetic neuropathy and the hope of ED treatment. The severity of ED was evaluated by a 5 item version of the international index of erectile function (IIEF-5). In the IIEF-5 score, 22~25, 17~21, 12~16, 8~11 and 5~7 points were judged normal, mild ED, mild~moderate ED, moderate ED and severe ED, respectively. The relevance of clinical factors to ED severity was examined with uni- and multivariate analyses.
**Results:** Data of 1,118 diabetic men was analyzed. The prevalence of mild and more advanced ED patients was 90 %. This percentage is double of the mild and more advanced ED prevalence in age-matched healthy men (43%). As a result of inquiring multivariate analysis using the proportion odds model, it was found that aging, hyperglycemia (HbA1 c>8.0%), insulin therapy, microangiopathy, hypertension, previous history of cerebro- or cardiovascular disease were risk factors for the progression of ED. Insomnia, anorexia, numbness or spontaneous pain of the both the feet tips and soles, diarrhea, constipation and dysuria were also related to the severity of ED. In contrast, hyperlipidemia was not a risk factor of ED. Approximately half of the diabetic patients who were diagnosed with mild or more advanced ED were interested in the ED treatment. In addition, one third of the 50~60-year-old patients with moderate~severe ED had the hope of the pharmacotherapy for their ED.
**Conclusion:** ED was found to be very common in Japanese male diabetics. Possible influences of both micro- and macroangiopathy on ED were suggested. Neuropathic symptoms (dysaesthesia of the legs, diarrhea and dysuria), anorexia and insomnia might become a factor in the diagnosis of ED in diabetics. Moderate ED patients in their middle age often have the hope of ED pharmacotherapy. These findings offer information which is useful for the improvement of the QOL of diabetic patients through ED treatment.

## 1036

**Impact of diabetes mellitus on the severity of erectile dysfunction and response to treatment: an integrated analysis of data from tadalafil clinical trials**

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**Background and aim:** Diabetes mellitus (DM) is associated with autonomic neuropathy, dyslipidemia, and endothelial dysfunction, all of which are risk factors for erectile dysfunction (ED). The risk of developing ED in men with DM is approximately 4-fold greater than in men without DM. We conducted a retrospective analysis of data from 12 placebo-controlled trials to characterize the efficacy and safety of tadalafil for the treatment of ED in men with DM compared to men without DM.
**Materials and methods:** Patients were randomized to tadalafil 10 mg, 20 mg, or placebo, taken as needed (up to one time per day) for 12 weeks (637 men with DM [mean 57 years] and 1681 men without DM [mean 56 years]). In men with DM, glycemic control was classified by baseline hemoglobin A1 c (HbA1 c) levels as good <7.0%, fair 7.0–9.5%, or poor >9.5%. Efficacy measures to assess erectile function included the International Index of Erectile Function erectile function (IIEF EF) domain score, Sexual Encounter Profile Diary Question 3 (SEP Q3, Did your erection last long enough to have successful intercourse?), and a Global Assessment Question (GAQ, Did the treatment improve your erections?).
**Results:** At baseline, men with DM had more severe ED than men without DM; mean IIEF EF scores were of 12.6 and 15.0, respectively ($p<0.001$). In men with DM, the mean baseline IIEF EF scores correlated inversely with baseline HbA1 c levels (good=14.1, fair=12.4, and poor=11.5; Pearson's r = −0.14; $p < 0.001$). Tadalafil significantly improved erectile function compared with placebo in men with ED both with and without DM ($p < 0.001$ for IIEF EF, SEP Q3, and GAQ). The mean improvement in IIEF EF score for men with DM was 6.2 for tadalafil 10 mg and 7.4 for tadalafil 20 mg, vs. 0.9 for placebo ($p < 0.001$ for both dose groups); and for men without DM, the mean change in IIEF EF was 6.7 for tadalafil 10 mg and 8.9 for tadalafil 20 mg, vs. 0.8 for placebo ($p < 0.001$ for both dose groups). In men with DM, the success rate of intercourse attempts (SEP Q3) was stable over time post-tadalafil dosing with mean per-patient percent success ranging between 50% to 63% over 0.5 to 36 hours post-tadalafil 20 mg dosing. The effect of tadalafil relative to placebo was not significantly influenced by baseline HbA1 c levels (interaction $p$-values of 0.52 for IIEF EF; 0.70 for SEP Q3). Additionally, tadalafil improved erectile function regardless of DM treatment therapy (i. e., insulin, oral DM medications, or neither; interaction $p$-values = 0.32 for IIEF EF and 0.90 for SEP Q3). Tadalafil 10 and 20 mg was well tolerated, with similar adverse events reported in men with and without DM. The most common treatment-emergent adverse events were headache, dyspepsia, back pain, myalgia, and nasopharyngitis. There was no increase in serious adverse events, including myocardial infarction or ischemia with tadalafil treatment. Less than 4% of patients in any group discontinued treatment due to adverse events.
**Conclusion:** This integrated analysis showed that men with DM have more severe ED than those without DM, and ED severity is related to glycemic control. Despite this, tadalafil was efficacious and well tolerated. The effect of tadalafil to improve erectile function was not significantly influenced by the patient's ongoing treatment for DM or HbA1 c levels.
*Supported by: LillyICOS LLC*

## 1037

**Efficacy and safety of two dosing regimens of Tadalafil in patients with diabetes and erectile dysfunction: SURE study in 14 European countries**

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**Background and aims:** Tadalafil is a phosphodiesterase 5 (PDE5) inhibitor that improves erectile function up to 36 hours post-dose in patients with erectile dysfunction (ED). Data from a subgroup of patients with diabetes mellitus in the SURE study were analyzed to evaluate the efficacy and safety of tadalafil when taken on demand or 3 times/week.
**Materials and methods:** SURE is a multicenter, crossover, open-label study investigating tadalafil for ED treatment in 14 European countries. 4262 patients, including 762 with diabetes, were randomized to treatment with tadalafil 20 mg, on demand (before sexual activity, maximum one dose per day) or 3 times/week, for 5–6 weeks. After a 1 week washout period, patients were crossed over to the alternate regimen for another 5–6 weeks.
**Results:** Of the 4262 patients randomized in SURE, 762 had diabetes and were included in this analysis. The mean age (SD) was 57 years (9.1) and the average BMI (SD) was 28.7 kg/m$^2$ (4.2). ED etiology was classified by the investigators as organic in 55%, mixed in 42%, and psychogenic in 3% of the patients. ED severity, determined using the IIEF EF domain, was severe in 43%, moderate in 28%, and mild in 29% of the patients. Some of the reported comorbidities included hypertension in 48%, hyperlipidemia in 22%, coronary artery disease in 6%, and depression in 5% of the patients. In these patients with diabetes, 57.2% preferred the on-demand regimen and 42.8% preferred the 3 times/week dosing.

**Efficacy and Safety**

| | Tadalafil dosing on demand (N=762) | Tadalafil dosing 3 times/week (N=762) |
|---|---|---|
| **Efficacy** | | |
| IIEF Erectile Function (EF) Domain (Mean endpoint score ± SE)* | 21.7 ± 0.3 | 22.0 ± 0.3 |
| Percent with Normal EF Domain Score (>=26) at Endpoint | 41.8% | 43.3% |
| Mean percent of attempts with successful penetration (SEP2 ± SE) | 73.0% ± 1.3 | 75.0% ± 1.3 |
| Mean percent of attempts with successful intercourse (SEP3 ± SE) | 58.0% ± 1.4 | 60.5% ± 1.5 |
| Number of Intercourse Attempts (%) | | |
| <4 hours from dose | 4442 (49.8%) | 2288 (27.6%) |
| >=4 hours from dose | 4479 (50.2%) | 6000 (72.4%) |
| **Safety**** | | |
| Dyspepsia | 45 (5.9%) | 44 (5.8%) |
| Headache | 36 (4.7%) | 43 (5.6%) |
| Backpain | 19 (2.5%) | 16 (2.1%) |
| Flushing | 12 (1.6%) | 16 (2.1%) |
| Myalgia | 11 (1.4%) | 15 (2.0%) |

* Baseline Mean IIEF EF Domain Score ± SE = 12.8 ± 0.5
** Treatment-Emergent Adverse Events >=2% incidence

**Conclusion** In the SURE study, a considerable number of patients with diabetes (43%) had severe ED of an organic or mixed origin and hypertension (48%) as a comorbidity. Most patients (57.2%) in this subgroup, as in the overall SURE population, preferred the on-demand regimen of tadalafil 20 mg while a large number (42.8%) preferred the 3 times/week dosing. Tadalafil was efficacious, as measured by the IIEF EF domain score and SEP 2 and 3, regardless of the treatment regimen preference, and a majority of these patients with diabetes had intercourse attempts more than 4 hours after taking the drug. Tadalafil was well tolerated with dyspepsia as the most common adverse event. In both the on-demand treatment and the 3 times/week dosing regimens, patients with ED and diabetes can benefit from the duration of effectiveness of tadalafil (up to 36 hours).
*Funding for this study was provided by Lilly ICOS LLC*

## 1038

**Efficacy and safety of sildenafil in relationship to the diabetes status in males with erectile dysfunction and diabetes mellitus**

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**Background and aims:** Erectile dysfunction is common in men with diabetes. Our objective is to evaluate efficacy, life satisfaction and safety of sildenafil in men with erectile dysfunction and diabetes mellitus, and the relationship between the response to study treatment and potential predictive factors such as diabetes complications.
**Materials and methods:** A double-blind (followed by open label treatment phase), randomized, placebo-controlled, parallel group, multicenter, flexible dose study, conducted October 2000 through June 2002 in Belgium and in the Netherlands.
Patients were randomized to sildenafil (107) or placebo (53) as needed, but no more than once daily, for 6 weeks, followed by open label treatment phase of another 6 weeks.
The primary efficacy variables are International Index of Erectile Function Questions 3 and 4, analyzed using an analysis of covariance (ANCOVA) model.
**Results:** 125 patients (78%) completed the study (82/107 in the sildenafil group, 43/53 in the placebo group).
By intention-to-treat analysis, at 6 weeks, patients receiving sildenafil were significantly better able to achieve erections compared with those receiving placebo (least square mean score 3,07 versus 2,25, p=0,002). The ability to maintain erections in the sildenafil group (mean score 2,71) compared with the placebo group (mean score 1,96) was also significantly better (p=0,005). The efficacy of sildenafil on achieving erections was significantly better (p=0,021) in patients with no complications of diabetes (mean score 3,04) compared to patients with one or more complications of diabetes mellitus (mean score 2,42), as well as the efficacy on maintaining erections (mean score 2,88 versus 2,06, p=0,003).
There was no difference in the efficacy of sildenafil between country (Belgium versus the Netherlands), age, duration of erectile dysfunction, etiology of erectile dysfunction, smoking status, BMI, diabetes control, cardiovascular complications, blood lipids, and blood pressure.
**Conclusion:** Sildenafil is an effective treatment for erectile dysfunction in men with diabetes mellitus, though in men with one or more complications of diabetes mellitus it seems less effective.
*The study was sponsored by Pfizer.*

# PS 102

# Diabetic foot: risk factors and epidemiology

## 1039

**Falls and its risk factors among elderly patients with diabetes**

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**Background and aims:** Study on falls and its risk factors among elderly patients with diabetes.
**Materials and methods:** A case-control study was conducted among 101 diabetic patients aged over 60 years older(69.01 ± 6.41 years older),comparing with 104 elderly people without diabetes(70.51 ± 5.89 years older).
**Results:** Falls in elderly diabetic patients (24.75%) were higher than elderly people without diabetes 13.4% (P<0.05).The number of elderly diabetic patients with hypertension, dyslipidemias, macrovascular, abnormal sight and hearing were higher than the number of elderly people without diabetes (P<0.001).The number of elderly diabetic patients with neuropathy disability: abnormal sensory tests included pain sense, topognosis , touch sensation, temperature perception, pressure sense and the absence of foot pulses were higher than the number of elderly people without diabetes (P<0.001).Univariate analysis showed that ten variables were significantly associated with a high risk for falls.By logistic regression analysis, six variables were identified as independent of each other. macrovascular, neurpathy and pressure sense, diabetic foot, orthostatic hypotention and hypotensor were associated with falls of elderly diabetic patients (P<0.05–0.01).
**Conclusion:** Diabetic neurpathy and peripheral vascular disease are the pathophysiologic basic on falls of elderly patients with diabetes.

## 1040

**Vascular risk markers may be more influential than glycaemic control in the development and progression of diabetic neuropathy – a retrospective analysis**

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**Background:** Peripheral neuropathy (PN) is a common yet poorly understood complication of diabetes mellitus (DM). We retrospectively studied the characteristics of patients with PN (vibration perception threshold (VPT) >25V).
**Methods:** Subjects who had PN in 2002 (i.e VPT >25V; n=100) and diabetic controls without PN (VPT<25V; n=200) were identified from our computer database (Proton®, CCL, UK). We studied the demographic and metabolic characteristics of those subjects attending our clinics on a yearly basis from 1995 to 2002.
**Results:** In 2002, PN subjects were older (p<0.001), had a longer duration of DM (p=0.01), higher serum creatinine (p<0.001) and higher BMI (p=0.04). They were more likely to have peripheral vascular disease (PVD; p<0.001). Mean VPT was higher in the PN group throughout the time period studied (p<0.001), and progressively increased over time. There was no change in the control group (p<0.001 for VPT rate of change). HbA1 c was also consistently higher in the PN group between 1995 and 2002 (p=0.01), although there was no difference in the rate of change. There were no differences between the groups over time in total cholesterol although rate of change did differ (p<0.001). In addition, triglyceride levels were consistently higher in the PN group (p<0.001), although the rate of change between 1995 and 2002 was different between the 2 groups (p=0.05). Systolic blood pressure (BP) was higher in the PN group (p<0.001) with no difference in rate of change, whilst diastolic BP was higher in the PN group (p =0.013), with different rates of change (p=0.007). Higher total cholesterol and diastolic BP were found to be independently associated with the presence of PN (p=0.001 and p=0.05 respectively).
**Conclusions:** These longitudinal data show that the rate of change of VPT in patients with PN is more accelerated compared to patients without this complication. Changes in glycaemic control seem to have little effect on VPT progression in patients with PN. Other modifiable risk factors (e.g. lipids, BP) may have a more influential role. Our findings support a vascular mechanism in the aetiology of PN.

## 1041

**Variability in activity may precede diabetic foot ulceration**

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**Background & Aims:** to evaluate the potential role of activity in development of diabetic foot ulceration.
**Methods:** We evaluated the first 100 consecutive evaluable persons (95.0% male, aged 68.5 ± 10.0 years with diabetes, neuropathy, deformity and/or a history of lower extremity ulceration/partial foot amputation) enrolled in an ongoing prospective longitudinal activity study. All patients were dispensed high-capacity continuous computerized activity monitors. Data were collected continuously over a minimum of 25 weeks (or until ulceration) with daily activity units expressed as mean ± standard deviation.
**Results:** Of the subjects enrolled, 8 ulcerated during the mean 37.1 ± 12.3 week follow-up period. Average daily activity was significantly lower in persons that ulcerated (U) compared to persons that did not ulcerate (NU) (809.0 ± 612.2 vs. 1394.5 ± 868.5, p = 0.03). Furthermore, there was a large difference in variability between groups. The coefficient of variation (CoV) was significantly greater in the U group compared with the NU (96.4 ± 50.3 vs. 44.7 ± 15.4%, p=0.0001). Furthermore, in the two weeks preceding the ulcerative event, the CoV increased even further (115.4 ± 43.0%, p = 0.02) but there was not a significant difference in average daily activity during that period (p = 0.5).
**Conclusions:** Persons with diabetes who develop ulceration may actually have a *lower* overall activity than their NU counterparts but the quality of that activity may be more *variable*. Perhaps modulating the "peaks and valleys" of activity in this population through some form of feedback might prove to reduce risk for ulceration in this very high risk population.
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## 1042

**Diabetic foot risk factors in diabetic patients with and without polyneuropathy. The North Catalonia Diabetes Study.**

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**Background and aims:** We studied the prevalence of Diabetic Polyneuropathy (DPN) and risk factors for developing diabetic foot in a cohort of type 2 diabetic patients (T2DM). The study was performed in three different regions with 92.912 inhabitants in the North Catalonia Region (North Catalonia Diabetes study NCDS).
**Materials and methods:** The sample was 307 subjects with T2DM (61.6% men), aged 59.63 ± 7.87 years, diabetic evolution (years): 8.6 ± 7, HbA1 c 7.0 % ± 1.44, BMI 30.01 ± 4.7, and 307 reference subjects, matched for sex and age (confidence interval: 95%). The diagnosis of DPN was realized by presence of two or more signs or one sign + two symptoms and several exploration was made: bilateral alteration of vibration perception thresholds (VPT) (with neurothesiometer and quantitative tuning fork), pinprick, cold, Semmes-Weinstein Monofilament (SW-MF) 5.07, reflexes, arthrokinesis and the items of modified Neuropathy Symptom Score. The diagnosis of *Peripheral (ischemia) vascular disease* was performed by the absence of foot pulses, claudication and by-pass record. It was also studied other different risk factors: extrinsic, intrinsic and favouring in diabetic patients. The study was evaluated in parallel in T2DM patients with and without DPN.
**Results:** DPN was observed in 23,17% of T2DM population. Polyneuropathy correlated to Systolic Hypertension (p<0.001) no to Diastolic and also was related to diabetic retinopathy, HbA1 c and HDL-c levels. The presence of retinopathy, Age, HbA1 c levels and HDL-c levels were used for study of polyneuropathy risk prevalence (Sensitivity of 74.2 % and specificity of 74–9 %). Peripheral ischemia was present in 4.56% (0.96% in reference sample). In table 1, It was expressed the different evaluation of polyneuropathy. All the studies were significant different between diabetic patients with DPN vs. without DPN. No differences were observed between reference group and T2DM without DPN except for VPT with tuning fork (p<0.01). Four variables (ankle reflex, VPT, SW monofilament and NSS

score) allow to establish a simplified method for the diagnosis DPN at primary care level with very high accuracy (Sensitivity 77.5% and specificity 94.9%. Correct assessment of 90.8 %)
**Conclusion:** Hypertension was not only related cardiovascular disease but also to diabetic polyneuropathy as another risk factor. The T2DM patients without DPN and the reference group did not manifested any differences in polyneuropathy evaluation except for the deep sensibility (VPT and a trend for the ankle reflex). Polyneuropathy diagnosis may be realize with four clinical variables at primary care level and polineuropathy risk could be made with other four variables. Several program models of computer may be useful in the quick diagnosis of diabetic polyneuropathy.

| | T2DM Sample With and Without Diabetic Polyneurpathy (DPN) | | | Reference Sample means and frequences related versus T2DM Sample with and with and out DPN. | | |
|---|---|---|---|---|---|---|
| VARIABLE | With DPN | Without DPN | p<Value | Non DM Sample | p< Vs. With DPN | p< Vs. Without DPN |
| Quantitative Tuning Fork | 3.46 | 5.61 | 0.001 | 5.90 | 0.001 | 0.01 |
| Neurothesiometer | 16.63 | 7.66 | 0.001 | 6.94 | 0.001 | 0.06 |
| SW 5.07 10gr. Monofilament | 7.17 | 9.63 | 0.001 | 9.62 | 0.001 | 0.852 |
| Ankle Reflex | 39.44% | 10.21% | 0.001 | 5.86% | 0.001 | 0.070 |
| Cold | 39.44% | 17.45% | 0.001 | 15.96% | 0.001 | 0.647 |
| Pin Prick | 33.80% | 4.66% | 0.001 | 4.58% | 0.001 | 0.962 |
| NSS modified scale | 3.72 | 2.17 | 0.001 | 2.05 | 0.001 | 0.616 |
| Ankle Dorsiflexion | 9.86% | 1.69% | 0.05 | 1.65% | 0.001 | 0.952 |
| Deformity | 32.39% | 20.76% | 0.063 | 26.38% | 0.308 | 0.124 |
| Peripheral Ischemia | 12.68% | 2.12% | 0.05 | 0.98% | 0.01 | 0.298 |

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## 1043

**Screening of high-risk diabetic foot: cohort-based study in Russia**
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**Background and aims.** To identify patients with high risk of foot ulcers and low extremity amputations (LEA) among out-patient diabetic population in three regions of St.-Petersburg in 2002–2003.
**Materials and methods:** Diabetic patients were assessed for medical history, 10-g monofilament, foot deformity and pedal pulse palpation. The modified Risk Classification System of the International Working Group on the Diabetic Foot was used for risk stratification. Risk of foot ulcer/amputation was categorized as "low" (no risk factors), "medium" (presence of neuropathy or foot deformity without angiopathy and history of foot ulcer or LEA), "high" (presence of neuropathy and foot deformity or angiopathy), and "extremely high" (history of LEA).
**Results:** 3807 diabetic patients were examined (95,5% - type 2 diabetes, 4,5% - type 1 diabetes). Male:female 854:2953. Mean duration of diabetes was 9,6 ± 3,1 yrs, BMI - 30,4 ± 8,2 kg/m$^2$. Mean age 66,5 ± 10,9 yrs. 76,8% were older than 60. The prevalence of risk degrees was as followed: low - 43%, medium - 37,3%, high - 14,6% and extremely high - 5% of pts. There were not discrepancies in age and BMI between risk groups but duration of diabetes was longer in patients with extremely high risk compared to low risk (14,9 ± 5,1 vs 8,4 ± 3,7 yrs, p<0.05). The major risk factors were foot deformities (46%). In 62 patients the foot ulcer was primary diagnosed during screening. Diabetic polyneuropathy and angiopathy were found in 11,5% and 10,6% patients, respectively. The distribution of risk degrees was different in type 1 and 2 diabetes. Low risk was identified in 56,6% and 42,5%; medium - 17,1% and 38,1%; high - 16,6% and 14,5%; extremely high - 9,7% and 4,9% for type 1 vs type 2, respectively (p<0.05). The prevalence of neuropathy, angiopathy, foot deformities and history of foot ulcer/amputation in type 1 diabetes was 22,8%, 15%, 30%, 8,2%. The corresponding data in patients with type 2 diabetes were 11,1%, 10,5%, 46,6%, 4,2% respectively (p<0.05 vs type 1).
**Conclusions:** The present study documented a high prevalence of patients with high and extremely high risk of ulcer/amputation in screened population. The distribution of risk groups and prevalence of risk factors seems to be different in types 1 and 2 of diabetes

## 1044

**Peripheral neuropathy may explain the distal distribution of atherosclerosis in the diabetic lower extremity**
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**Background and aims:** The distal distribution of arterial occlusive disease is unexplained in diabetes. We hypothesised that neuropathy may lead to medial calcification and then plaque formation. The aim of the study was to investigate the relationship between axonal neuropathy in the distal nerves (sural/tibial) and medial wall calcification, arterial plaque formation and thrombosis in the tibial arteries in amputated limbs.
**Materials and methods:** We studied two groups of patients. Group 1 consisted of 38 diabetic patients (mean age: 67 ± 14 years, mean±SD) and group 2 consisted of 34 non-diabetic controls (mean age: 58 ± 20 years). (p=NS, non-significant).
Medial wall calcification, plaque formation and thrombosis as well as axonal fibre loss were assessed semi-quantitatively and scored from 0 to 3 (0 - no disease, 1 - mild, 2 - moderate, 3 - severe).
**Results:** Axonal neuropathy score was markedly increased in group 1 compared with group 2 (Group 1: 2.84 ± 0.35 vs Group 2: 0.36 ± 0.82, p<0.001). Tibial arterial disease in the diabetic group was confirmed by increased medial wall calcification score (Group 1: 1.90 ± 1.14 vs Group 2: 0.29 ± 0.61, p<0.001) and plaque formation score (Group 1: 2.08 ± 1.02 vs Group 2: 1.20 ± 1.34, p=0.003) but not increased thrombus formation score (Group 1: 1.12 ± 1.10 vs Group 2: 0.85 ± 1.27, p=NS).
Overall, there was a significant correlation of neuropathy with calcification (rs= 0.538, p=0.0001; rs-Spearman's rank correlation coefficient) and of neuropathy with plaque formation (rs=0.384, p=0.001). Plaque formation was also correlated with medial wall calcification (rs=0.310, p=0.008) and with age (rs =0.585, p=0.0001). Thrombus formation was correlated with age only (rs =0.437, p=0.001). There was no association of neuropathy with thrombosis (rs=0.154, p=0.213)
**Conclusion:** Neuropathy was significantly correlated with both medial wall calcification and plaque formation, which in itself was correlated with medial wall calcification. Thus, peripheral neuropathy may lead to medial wall calcification and then plaque formation in the tibial arteries and this may explain the distal distribution of atherosclerosis in the diabetic lower extremity.

## 1045

**Decreased amputation rate in diabetic patients. An observational study in a multidisciplinary diabetic foot clinic**
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**Backgrounds and aims:** From 1998 to 2003 the amount of patients with diabetic foot ulcers seen in diabetic outpatient clinic were doubled, due primarily to an increase in type 2 diabetic patients. The total numbers of patients seen in the foot clinic was about 2.700. The aim was to identify risk factors in patients undergoing amputations, and to review the value of a multidisciplinary setting consisting of podiatrist, orthopaedic surgeon, diabetes specialist and nurse.
**Material and methods:** All journals of amputated diabetic patients seen in the multidisciplinary diabetic foot clinic in the 6 years period from 1998 to 2003.
**Results:** 120 diabetic patients underwent 140 amputations. Number of patients with above and under knee amputations was halved from 6 to 3 per year (p 0,01) in the observational period leading to an incidence of 0,03% diabetic patients or 1,7 of 100.000 inhabitants. Number of patients with minor amputations was 10,3 ± 2,5 per year with no increase in the period. 40 % of the patients were reamputated 1–2 times during the next few years. The localisations of amputations (total of 140) were toes 47%, transmetatarsal 19%, under knee 27% and above knee 9%. All patients had foot ulcers, severe neuropathy and 2/3 had moderate to severe decreased blood support to the foot. Nearly all patients with above and under knee amputations had severe ischaemia. About 2/3 of the patients attended the foot clinic at the time of the first amputation. In patients who were regularly seen in the clinic before and after the amputation, none had isolated neuropathy and the amputating rate were halved compared with patients seen at the time of amputation. A reduced amputation rate in the group followed after amputation could not be shown maybe due to a short time course. The mean $HgA_1c$ was 9,0% and LDL-cholesterol 3,3 mmol/l with no significant difference between the patients seen or not seen before the first amputation. Most patients have several severe complications e. g. AMI, stroke, retinopathy and nephropathy.

**Conclusion:** Despite the increasing amount of type 2 diabetic patients, it is possible to reduce the number of patients being amputated and to reduce the reamputating rate in a multidisciplinary diabetic foot clinic consisting of podiatrist, orthopaedic surgeon, diabetes specialist and nurse. Important risk factors are foot ulcers, foot deformities, neuropathy, bad metabolic control and ischaemia, which have to be treated aggressively to avoid invalidating amputations.

## 1046

**Limb preserving amputation outcomes among the diabetic population at Madigan Army Medical Center in Tacoma, Washington, U.S.A.**

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**Background and aims:** 18.2 million people, or 6.3% of the population, in the USA have diabetes. More than 60% of non-traumatic lower extremity amputations occur among diabetic patients. The 3-yr survival rate after one major lower extremity amputation is 50%, and the 5-yr survival rate is 40%. The survival rate is related to continuing disease, but more importantly to the associated increased metabolic cost of a major lower extremity amputation. To increase the survival rate, by decreasing the metabolic cost to the diabetic patient, limb preserving amputations such as toe, midfoot and/or isolated rearfoot amputations should be considered. The goal of the Limb Preservation Service at Madigan Army Medical Center is to utilize surgical procedures that resect infected soft tissue and bone, while maintaining a functional foot.
**Materials and methods:** The outcomes data was assessed, by defining diabetes by any concomitant 250 ICD-9 code and amputations by 84.1–81.18 procedure codes.
**Results:** The CDC lower extremity amputation rates for the year 2000 are 12% for above knee, 30% for below knee, 14% for foot and 44% for toe. At Madigan Army Medical Center the average lower extremity amputation rates for 1998–2003 are 10% for above knee, 20% for below knee, 20% for foot and 50% for toe. The incidence rate of lower extremity amputations at Madigan Army Medical Center has decreased from 9.9 per 1000 in 1999 to 1.8 per 1000 in 2003, while the number of diabetic patients has increased from 3343 in 1999 to 4936 in 2003.
**Conclusion:** The reduction in major lower extremity amputations and the increase in limb preserving amputations, such as toe, midfoot and/or isolated rearfoot should be considered a viable surgical option to increase the survival rate of the diabetic patient.

# PS 103

# Diabetic foot: management and Charcot foot

## 1047

**Neuropathy, osteoporosis and vascular calcification – do RANK-L and osteoprotegerin provide the missing link?**

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**Background and aims:** Neuropathy is associated with an increased incidence of calcification of the arterial wall in diabetes. Neuropathy is also associated with osteoporosis in diabetes. Both vascular calcification and osteoporosis are at their most marked in acute neuropathic osteoarthropathy (acute Charcot foot). The mechanism linking these associations has been obscure, although it has been thought it may result from loss of innervation of vascular smooth muscle with resultant abnormalities in intravascular flow and pressure. However, the recent discovery that the RANK-L and osteoprotegerin (OPG) cytokine pathway is central to the activation of osteoclasts and bone breakdown in many different conditions has raised the possibility of its involvement in diabetic neuropathy. This possibility is made more likely by the fact that the RANK-L/OPG pathway is also involved in the development of calcification in vascular smooth muscle cells. RANK-L and OPG have essentially opposite actions: RANK-L triggers both the maturation of osteoclasts and vascular calcification, while the role of OPG is essentially compensatory and protective. Nevertheless, the circulating conentrations of both are elevated in conditions associated with increased osteolysis.
**Materials and methods:** We have therefore undertaken a preliminary study of circulating OPG (Quidel®) in the peripheral blood of patients (age 37–73; 23M,13F) with diabetes (N=26), with (N=20) and without (N=6) distal symmetrical neuropathy, and healthy controls (N=10). RANK-L results are not given since the majority were below the sensitivity of the assay used.
**Results:** Median (5–95% CI)) fasting serum concentration of OPG was 6.05 pmol/L (2.59–36.07) and was significantly higher than controls (3.30; 1.60–5.50), P=0.001 (Mann-Whitney). Despite the small numbers, the difference observed between diabetics with neuropathy (6.40, 3.90–45.70) were significantly higher than in those without (4.55, 2.10–6.40), P=0.046.
**Conclusion:** These preliminary data suggest an association between the RANK-L/OPG signalling system and the pathogenesis of morphological complications observed in diabetic neuropathy.

## 1048

**Treatment of severe foot instability in Charcot foot of the ankle with intramedullary nailing compressive device**

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**Background and aims:** The involvement of the ankle in the chronic Charcot foot leads to a fracture of the astragalus responsible for severe deformity and recalcitrant ulceration over the hip of the fibula with high risk of major amputation. The treatment is the ankle artrhodesis that pay a high risk of non-union with classic synthesis devices.
**Materials and methods:** In the period from January 2001 to December 2002 we have applied the intramedullary nailing compressive device in 14 diabetic patients affected by Charcot of the ankle: in all the patients a below-knee amputation were suggested. Four patients suffered from an ulcer, healed before surgical treatment
**Results:** In a follow up period of 184 months 10 patients (71,4%) have reached a solid artrhodesis, returning to normal walking with protective shoes while 3 patients (21,4%) had hardware complications (breaking of calcaneous screws with need of removal of the screws in two cases and removal of the entire nail in two cases) with evidence of fibrous union that allowed walking with tutor. In 1 patient (7,2%) a below-knee amputation was performed due to the postoperative evidence of osteomyelitis of the distal party of the tibia
**Conclusion:** The data from our study that show a high rate of limb savage (92,8%) allow us to underline that this synthesis device is safe and effective.

| Population Data | |
|---|---|
| n. patients | 14 |
| sex | 13 / M 1 / F |
| age | 58 ± 12 |
| type of DM | 12 / type 2 2 / type 1 |
| Duration of DM | 17 ± 5 |
| Treatment | 12 / ins 2 / OH |
| VPT | 15 />25 bilateral |
| Monofilament 10 gr | 15 />0/6 |
| TcP02 | 14 />50 mmhg |
| Pedal pulses | + 14 /14 |
| Previous Foot Ulcer | 4 /14 |

Outcomes of ankle arthodesis (follow-up 18 4 months)

| | Total | Not previous ulcer | Previous ulcer |
|---|---|---|---|
| n. patients | 14 | 10 | 4 |
| Solid arthrodesis | 10/14 (71,4%) | 10/10 (100%) | 0 (0%) |
| Fibrous union | 3/14 (21,4%) | 0 | 3/4 (75%) |
| Hardware complication | 4/14 (28,6 | 0 | 4/4 (100%) |
| Protective Shoes | 10/14 (71.4%) | 10/10(100%) | 0 (0%) |
| Walking brace | 3/14 (21.4%) | 0 | 3/4 (75%) |
| Major amputation | 1/14 (7.2%) | 0 | 3/4 (75%) |

## 1049

**Evaluation of a new indicator plaster in identifying diabetic patients at risk of foot ulceration**

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**Background and aims:** Peripheral autonomic neuropathy combined with sensorimotor neuropathy leads to the foot ulceration as the insensitive dry skin often cracks resulting in minor trauma. A new indicator plaster (Neuropad) changes its color if moisture is present and the hypothesis is that stability of the color could indicate absence of sweating as a result of peripheral autonomic nerve dysfunction.
**Materials and methods:** To test this hypothesis 74 type 2 diabetic patients were examined (males = 34), mean age and mean duration of diabetes were 65,74 ± 9,25 and 16,92 ± 9,51 (yrs) respectively. Motor and sensory deficits were assessed in both legs and scored using a modified scoring system of this proposed by P. J. Dyck – Neuropathy Disability Score -NDS – (tendon reflexes -maximum 8- and reduced sensation of pain, cold, touch and vibration -maximum 20-). For the diagnosis of small fiber dysfunction (e. g. reduced pain, touch and cold sensation) the NDS1 was used as the sum of these scored sensory deficits and for this of large fiber dysfunction the score of reduced vibration sensation. For the statistical analysis the chi-square test and the multiple regression stepwise model were used. The overall predictive values (positive and negative -PV) for nerve function using this plaster were assessed in all the cases.
**Results:** a) In bivariate analysis 79,07% of patients with full change in the plaster's color (Neuropad negative-NN) didn't reveal any significant nerve dysfunction and 58% of patients with partial change or stability of its color (Neuropad positive-NP) showed such a dysfunction (NDS>3)- $p<0,05$ and the PV was 68.92%. Small fiber dysfunction (NDS1>2) was established in 61, 29% of NP patients and no significant deficits (NDS1≤2) or their normal function was established in 76, 74% of NN patients, $p<0, 05$ , since the PV was in this case 70.27%. Large fiber dysfunction was present in 58% of the NP patients and normal function of these fibers was in 79% of the NN patients ($p<0,05$ ) and the PV was 70%. b) In the multiple regression analysis the following parameters a) NDS, b) NDS1, c) large fiber dysfunction and d) duration of diabetes were significant factors for Neuropad positive or negative results, whereas those reflecting nerve dysfunction were the most powerful.
**Conclusion:** Stability or partial change of the color of the new plaster (Neuropad) is significantly dependent of peripheral autonomic and somatic nerve dysfunction and could identify in daily practice a significant part of patients at risk of foot ulceration

## 1050

**A comparative study between color change plaster for diabetic foot syndrome and electrical impedance change of plantar skin sweat secretion**

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**Background and aims:** Lower extremity amputations are the most prevalent and costly of all the diabetes-related complications. We aimed to asses the value of a color change plaster for detecting diabetics who show a high risk of developing neuropathic foot ulcers and to develop a method to store clinical data and digital images to build a secure standardised database.
**Material and methods:** For diagnostic of diabetic foot syndrome and peripheral neuropathy, based on detection of the plantar site sweat secretion, we have developed a comparative study between color change plaster-Neuropad$^R$ for diabetes foot syndrome (blue=abnormal, blue/pink=borderline or pink=normal) and the electrical impedance changes induced by the skin sympathetic sudomotor activity (SSA) detected with an Impedance Reactometer$^R$, a PC-based system developed in our laboratory. This system is based on a self-balancing technique and a lock-in detection, responding to small changes of electro dermal parameters induced by activation of ecrine sweat glands to muscarinic cholinergic agents. Skin impedance fluctuations around equilibrium reflect the dynamics of the functional state of SSA under resting condition or during stimulus induced responses. The AC current, inversely proportional to local skin impedance, is converted to an ac voltage. New software and automated analysis programs were developed for the clinical research. A PowerShot A70 battery-operated with minimal adjustment of the default settings digital camera captured directly JPEG images of the feet at a resolution of 2048 × 1536 pixels with 24-bit colour. Images were stored with patients' data, standardised on the WHO/Europe recommended Basic Information Sheet diabetes dataset and were collected in the Black Sea Tele Diab system an EPR system.
**Results:** We assesed sweat secretion of plantar site both feet on 34 diabetics (88% type 2 and 12% type 1) aged between 31and 65 years with an average duration of diabetes of 17 years. Using our technique we have shown that the skin sympathetic activity, expressed in mV, has much lower amplitude according to the functional state of SSA due to diabetic neuropathy. A significant correlation could be observed between Neuropad and measurements of impedance changes induced by the SSA which confirmed the relationship between Neuropad and Impedance Reactometer: on 12 diabetics with neuropathy (without foot ulceration) ($r=0.53, p<0.001$), on 14 patients at borderline ($r=0.67$, $p<0.001$) and on 8 patients without neuropathy ($r=86.3\%$ $p<0.0001$). These results have indicated that the Neuropad and Impedance Reactometer are equally suitable of use in the diagnosis of the skin sweat secretion impairment at plantar site in diabetes. However, the Neuropad is less expensive than the Reactometer and it is suitable for the patient self-examination use as a control routine examination every 6 months or diagnostic test for medical care. The findings were pathological if both of the examination methods demonstrated the result of disturbed or absence of the sweat secretion and plantar insensitivity to pressure from the 10g-monofilament. Prospective store of clinical data and digital images will be used to monitor the health care outcomes of patients with diabetes.
**Conclusion:** These preliminary results suggest that colour change plaster for diabetes foot syndrome and the impedance changes induced by the SSA significantly correlate and may be negative prognostic impact of neuropathic foot ulcers.

## 1051

**Efficacy of percutaneus transluminal angioplasty in diabetic patients with ischemic foot ulcers**

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**Background and aims:** To analyze efficacy of percutaneous transluminal angioplasty (PTA) of lower extremity arteries in diabetic patients with ischemic foot ulcers.
**Materials and methods:** 30 diabetic patients with non-healed foot ulcers were selected on basis of signs of critical limb ischemia (transcutaneous oxygen tension at the dorsum of the foot < 30 mm Hg, ankle-brachial index < 0,6). In all subjects angiography was carried out. 20 of them (11 men, 9 women) were feasible for PTA. Average age was 64 yrs (48–72), duration of diabetes - 12 (2–30) yrs, 2 patients were on chronic hemodialysis. 24 stenoses and 22 occlusions were dilated (46 interventions in 20 patients):

3 (7%) external iliac arteries (EIA), 10 (22%) superficial femoral arteries (SFA), 8 (17%) popliteal arteries (PA), 25 (54%) crural arteries. Stents were used in 100% of EIA, 60% of SFA, 25% of PA and 28% of crural arteries interventions. Average occlusion length was 4.9 (2–8) cm.
**Results:** In 5 (25%) patients ulcers healed in 2–12 weeks; in 10 (50%) patients minor amputations were performed; 3 (15%) underwent major (all below knee) amputations. In 5 patients after minor amputation wound was closed with a skin graft. 2 patients died: 1 myocardial infarction on 14th day and 1 stroke on 2nd day. Average follow-up time was 2 yrs (1 month- 3.5 yrs): 1 major (above knee) amputation was performed in this period (due to progression of vascular disease and non feasibility for revascularisation).
**Conclusion:** Distal pattern of vascular lesions are common for diabetic patients. Our experience proves that the PTA is feasible in majority of diabetic patients with critical limb ischemia, even in presence of extensive stenoses or occlusion. Such limb-saving angioplasty allows to avoid major amputation in most of cases with ischemic foot ulcers.

## 1052

**The Biogun® a novel way of eradicating MRS colonization in diabetic foot ulcers**

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**Background and aims:** There is increasing evidence that MRSA colonization of chronic foot ulcers is associated with an increased risk of bacteraemia, invasive intervention and prolonged ulcer healing time. The Biogun is a device which produces a concentrated stream of superoxide anions ($O^{2-}$) which disrupt the bacterial phospholipid bilayer causing cell lysis. The Biogun has been used by dentist to treat dental carries and podiatrist to treat verruca. In vitro study has shown it to be effective against MRSA. Is the Biogun effective at eradicating MRSA colonisation in diabetic foot ulcers?
**Material and methods**: This is a prospective study of fifteen consecutive patients with diabetic foot ulceration with no evidence of infection clinically but is colonized with MRSA attending a specialized diabetic foot clinic. All patients receive standard treatment with debridement by a podiatrist and appropriate off loading. All patients received topical MRSA eradication as per hospital protocol for colonisation and treatment with the Biogun. The probe was placed 2–10 mm above the ulcer surface and at a speed of not less than 60s/cm$^2$. Patient was treated until MRSA was eradicated or up to a maximum of 3 times with each episode at least 2 days after completion of the standard hospital MRSA eradication program. MRSA swab was taken from the wound after debridement of superficial exudates using sterile instruments and MRSA swabs also taken form hairline, nostril, throat, axilla, and the perineum. MRSA clearance was defined as 3 consecutive swab negative for MRSA.
**Results:** There was a 60% successful eradication of MRSA colonisation with the majority, 6 requiring only one course and 3 requiring a second course of treatment. There were no significant differences between the groups that had successful MRSA eradication and those that were unsuccessful in term of age, type of diabetes, duration of diabetes, duration of the foot ulceration or the proportion of neuroishaemic ulcers. There was a significant difference in the HbA1 c between the two groups although the MRSA persistent group had a better HbA1 c of 7.8% compare to 9.9% for the successfully treated goup ($p<0.05$). The only factor that influenced the success of treatment is the ulcer size where MRSA eradication was unsuccessful being significantly larger than the successful group, $843.3 \pm 254.4\,mm^2$ vs. $294.8 \pm 104.6\,mm^2$ ($p<0.05$). Surprisingly there was no significant difference between the duration that the foot ulceration was colonised with MRSA and the success of MRSA eradication although the success of MRSA eradication at non ulceration sites almost reaches significance ($p=0.06$).
**Conclusion:** Using the Biogun it is possible to eradicate MRSA colonisation in clinically uninfected diabetic foot ulcers in the majority of patients after only 2 courses of treatment. We have previously reported the mean duration of MRSA colonisation in infected foot ulceration was 28.3 weeks. For MRSA colonisation of non infected foot ulcers using the Biogun this has now been reduced to less than 4 weeks for most of our patients. These results are promising but a larger randomised placebo controlled study is required to confirm these results. The duration and frequency of treatment still need to be optimised and a more efficient method of delivering the superoxide need to be developed to make it a viable method of MRSA eradication in the routine clinic.

## 1053

**Effects of local treatment with a protease-inhibitor on cellular expression of matrix-metalloproteases in diabetic foot lesions**

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**Background and aims:** Wound healing in diabetes is impaired and non-healing ulcerations are a major health problem. To understand wound healing at the molecular level is a major focus of research. Persistent high levels of matrix-metalloproteases (MMP's) are relevant factors for wound chronification. The topical use of protease-inhibitors may affect the wound healing and promote the transition from a chronic to an acute wound.
**Materials and methods:** We included 18 patients with chronic diabetic foot lesions (stadium Wagner 2) in this study. Six patients received standard "good wound care", 12 Patients were additionally treated with a protease-inhibitor (Promogran®, Ethicon) and the dressings were changed daily. At the first visit and after five days a 3 mm punch biopsies were taken from the center of the wound and immediately frozen at -20°C. All samples were analysed by zymography for MMP-2 pro, MMP-2 active, MMP-9 pro and the dimer form
**Results:** Levels of MMP-2 and MMP-9 were not different between both groups before treatment. For MMP-2 active a significant reduction in the protease-inhibitor treated group after five days ($p=0.012$) was found. MMP-2active was clearly increased in the group treated with "good wound care" alone (9004 vs. 17022 pg/ml). The data for MMP-2 pro and MMP-9 (pro and dimer form) were not statistically different between both treatment groups and at the four different time points.
**Conclusion:** The local treatment with a protease-inhibitor beneficially affects clinical wound healing. In previous presented data we demonstrated unchanged mRNA levels of MMP's during treatment with a protease-inhibitor and at the level of cell-tissue.
In contrast to this a clear reduction of the biological active MMP-2 in proteases-inhibitor treated wounds was found.
Similar effects on MMP-2pro and MMP-9 (pro and dimer forms) were not found in this pilot study.
Local treatment with proteases-inhibitors does not affect the expression on mRNA or cell levels but seems to modulate the MMP's in the wound fluid. This could lead to lower active MMP levels on the surface of the wounds with reduced local proteolytic effects and improved wound healing.
*This study was funded by Johnson & Johnson*

## 1054

**Autologous graft of platelets obtained by plasma-apheresis in the management of neuro-ischaemic lesions of the diabetic foot**

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**Background and aims:** To test the safety and effectiveness of application of autologous platelets and their derivatives obtained by apheresis in the management of neuroischaemic ulcers of the diabetic foot.
**Materials and methods:** We studied 10 patients (2DM1/8DM2, Aged $69.2 \pm 8.2$ yrs, Diabetes Duration $29.4 \pm 10.3$ yrs, HbA1 c $7.9 \pm 1.8\%$, $TcPO_2$ $12.2 \pm 2.4$ mmHg) with lesions lasting more than 8 weeks, compared with a matched control group conventionally treated. 300 ml blood was withdrawan to obtain a platelet concentrate preparation using the „Buffy-Coat" technique on a Haemanetics Plus separator. The cellularity of the final preparation was 2–4x10$^6$, while 100 ml cryoprecipitate was obtained and split in 4 – 8 fractions. Autologous thrombin from coagulated serum of the same patient was used as activator. In the active group, the lesions were debrided, photographed and measured before each of 4–8 application. To this purpose, gel obtained upon activation was seeded on esterified hyaluronic acid sheets (Hyaff®, FAB, Abano Terme, Italy) applied onto the ulcers. After three days the gel was removed and substituted with Hyaff® alone. Control group patients underwent the same procedure with the exception that only Hyaff® was applied on the lesions.
**Results:** During the 4-month follow-up, 4 out of 12 ulcers healed in the active group, while only one out of 12 lesions healed in the control group ($p<0.01$). Mean lesion area reduction was 25% in the study group and 15% in the control group ($p<0.01$). At the end of the 4-mo follow-up, the granulation area was greater in the active than in control group ($23.8 \pm 9.9$ vs $10.4 \pm 5.3\,cm^2$; $p<0.001$). No adverse effect was recorded nor in the sampling and preparation of the gel nor in the application phase.

**Conclusion:** Autologous platelet gel obtained by plasma-apheresis is safe and more effective than advanced dressing alone in the management of neuroischaemic ulcers of the diabetic foot.

## 1055

**How often do heel ulcers heal in diabetes?**

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**Background and aims:** Ulcers of the diabetic heel present a difficult clinical challenge, especially in the elderly and in those with co-morbidities. There are no clear guidelines for management and they often appear to persist unaltered despite treatment. There has, however, been no previous attempt to document their outcome systematically, and to explore whether the rate of healing is related to ulcer type. We have therefore examined the experience gained in the management of heel ulcers in a single specialist clinic.
**Materials and methods:** A total of 157 heel ulcers (from 97 people, 121 legs) were first assessed in the 47 months between 1st January 2000 and 30th November 2003; 3 were excluded from analysis because of osteomyelitis diagnosed at presentation. The remaining 154 were categorised according to area, depth, infection, ischaemia and neuropathy and outcome.
**Results:** 101 of the 154 (65.6%) healed after a median (range) of 200 (24–1225) days. 30 (19.5%) remained unhealed at the time of death; 11 (7.1%) had resulted in an amputation and 12 (7.8%) remain unhealed. Significant differences in outcome were observed between those with different degrees of ischaemia, with 82.8% of 29 with palpable pulses, 79.3% of 29 with palpable but diminished pulses, and 56.3% of 96 with moderate or severe ischaemia healing, respectively (p=0.001, Mann-Whitney U). Significant differences were also observed between ulcers of different area ($<1\,cm^2$, $1–3\,cm^2$, $>3\,cm^2$), with 76.8% of 82, 59.5% of 37 and 43.8% of 32 healing, respectively (p=0.013, Mann-Whitney U). No differences were observed between healing and the presence of either slough or clinical infection (p=0.145, chi-squared), ulcers of different depth (p=0.051, Mann-Whitney U) or degrees of neuropathy (p=0.596, Mann-Whitney U). Logistic regression suggested that 70.1% of healing could be predicted from ischaemia and area.
**Conclusion:** These data indicate that the outcome of heel ulcers is more favorable than is generally believed, and also provide data which can be used as the basis for informed discussion of management options with the patient and their carers.

# PS 104
# Clinical retinopathy

## 1056

**Automated retinal image differencing**

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**Background and aims:** The National Diabetes Programme specifies an annual screening for retinopathy. This programme designates digital photography as the optimal way of achieving auditable and high-quality screening. Images can be rapidly stored, retrieved and logically catalogued. However skilled ophthalmology-trained personnel are still required to examine the images. The current estimate is that there are 48 million people in Europe with diabetes, and for two eyes and two or more fields of vision, this equates to 192 million images annually requiring an opinion. One alternative is computer automation, but lesion recognition in digital fundal photographs is problematic. There are major issues relating to sensitivity and specificity and lesion recognition programs are not yet robust enough for clinical use.
**Materials and methods:** A.R.I.D. or Automated Retinal Image Differencing is a computer system being developed that uses alpha blending transparency-differencing technology to detect and display change in images from one visit to the next.
**Results:** When ARID operates on two retinal images with no retinopathy it detects a change of 0.59% or a concordance of 99.41%. It performs a differencing using the Hue, saturation and Lightness colour model and is able to produce an image of change. When processing an image that has been manually manipulated, by a 15-degree rotation, the concordance achieved was 98.83%. In an image pair where proliferative retinopathy was present in one image but not the other, a change of 2.19% was detected. In visual terms this shows as a retinal image with ghosting of the arcades, and a clear prominence of new vessels. The program performs registration and differencing of images of size 432x 324 in 0.8 seconds. The aim is to have a real time differenced image available when subsequent fundal images are captured, to aid an ophthalmologist visually to analyse change between visits. Initial images will not difference successfully without image registration, and subsequent photographs require translation, rotational, magnification, astigmatic, clouding, intensity, brightness and colour analysis and adjustment.
**Conclusion:** A.R.I.D currently solves three of the registration problems (translation, rotation and colour analysis/adjustment) and is able to differences these registered images. This initial work is being expanded to establish a validated computing program

## 1057

**Clinical risk factors and VDR gene polymorhisms role in diabetic retinopathy in Polish Type 2 diabetes patients**

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**Background and aims:** Evidence exists that clinical, metabolic, and genetic risk factors are associated with the development of diabetic retinopathy. The aims of the study were: 1) To define the prevalence of diabetic retinopathy in patients with type 2 diabetes mellitus (T2DM) in a Polish population. 2) To analyze the clinical features associated with this complication in the examined group. 3) To search for the association of 4 markers of the vitamin D receptor (VDR), a candidate gene for vascular complications in diabetes.
**Materials and methods:** We included 267 T2DM patients in the study (female/male: 146:121, age at examination: 61.2 +/– 12.4 years, age at T2DM diagnosis: 50.0 +/– 9.2, T2DM duration: 11.6 +/– 7.8 years, body mass index (BMI): 30.5 +/– 5.5 $kg/m^2$, $HbA_{1c}$: 7.8 +/– 1.5 %). In all patients, clinical and metabolic profiles were determined. The stages of retinopathy were defined by a trained ophthalmologist by ophtalmoscopy after pupillary dilatation. Colorful photographic documentation was made. The examined T2DM patients were genotyped for FokI, ApaI, BsmI, and TaqI, which are frequent VDR polymorphisms based on the restriction fragment length polymorphism method. The statistical analysis was performed using univariate and multivariate logistic regression (SAS) and haplotype analysis (Haploscore).

**Results:** Diabetic retinopathy of different stages was detected in 85 (31,8%) patients with T2DM. The multivariate analysis revealed that significant predictors of diabetic retinopathy were: never-smoking status (p=0.0007), urea serum level (p=0.004), HbA1 c level (p=0.04), and insulin treatment (p=0.04). The other features such as age at time of T2DM diagnosis, BMI, coronary artery disease, arterial hypertension, and T2DM duration prior to ophthalmic exam were univariate predictors of diabetic retinopathy, however, lost significance as independent predictors in multivariate analysis. Similarly, the alleles, genotypes, haplotypes, and haplotype combinations of the VDR gene were not associated with the examined complication. However, there was a suggestion of a possible slight association between the fbaT haplotype and retinopathy (p=0.11).
**Conclusion:** Our study showed that diabetic retinopathy in T2DM patients remains a frequent complication in a Polish population. We were able to confirm the role of some clinical risk factors, surprisingly including not-smoking status as it was previously shown in UKPDS study. To define the role of the VDR gene in a Polish population, examination of a larger set of patients is required.

## 1058

**Six-year incidence and progression of diabetic retinopathy: results from the Mauritius Diabetes Complications Study**

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**Background and aims:** Diabetic retinopathy is a leading cause of visual loss throughout the world. Here, we determine the six-year incidence and progression of diabetic retinopathy in the multiethnic population of Mauritius and examine risk factors for retinopathy.
**Materials and methods:** A longitudinal population-based study was conducted during 1987, 1992 and 1998, in Mauritius (response rates: 86%, 87% and 87% respectively). Participants identified through the study as having diabetes (both known and newly diagnosed, by self-report and oral glucose tolerance test) and one in four with impaired glucose tolerance (IGT) underwent complications screening in 1992 and 1998. Retinal photographs were taken using a TRC-50VT retinal camera.
**Results:** Results were obtained from 339 participants with data available at follow-up (261 participants were free of retinopathy at baseline). The six-year incidence of diabetic retinopathy was 23.8% (sight-threatening in 0.4%). Among those with previously diagnosed diabetes (KDM) and free of retinopathy at baseline the incidence of non proliferative diabetic retinopathy (NPDR) was 29.2% and proliferative diabetic retinopathy (PDR) was 1.0%. Among those with NDM at baseline the incidence of NPDR was 19.1% (no incident cases of PDR were found). No incident cases of retinopathy were evident among those with IGT at baseline. Among those with mild NPDR at baseline (NDM & KDM) 46.6% at follow-up had no evidence of retinopathy and 27.7% had progressed at least one step, of whom 5.2% had developed PDR. Of those with moderate NPDR at baseline, 35.3% had progressed at least one step, of whom 29.4% had developed PDR. All participants with PDR at follow-up had undergone laser treatment. Independent risk factors for incident retinopathy (NPDR and PDR) were duration of diabetes and fasting plasma glucose.
**Conclusion:** This is one of the few recent population-based studies of diabetic retinopathy to be undertaken in a developing nation. The incidence of retinopathy in Mauritius was similar to that reported in developed countries, and was high among those with NDM at baseline. Progression of retinopathy was strongly and positively related to baseline severity of retinopathy.
*R.J. Tapp is supported by scholarships from the National Health and Medical Research Council of Australia and the Victorian Health Promotion Foundation*

## 1059

**Pulse pressure above 50mmHg is associated with increased risk of diabetic retinopathy; preliminary results of a retrospective cohort study**

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**Background and aims:** In diabetic patients, pulse pressure is an independent cardiovascular risk factor which has also been shown to be related to diabetic retinopathy in type 1 diabetic individuals. The aim of this study was to determine whether pulse pressure (PP) is associated with diabetic retinopathy.
**Materials and methods:** All diabetes outpatient clinic records of a state hospital between 1995 and 2003 were retrospectively screened for metabolic (A1 c in %, urinary albumin excretion rate UAER in mg/day) and clinical (systolic- and diastolic blood pressure in mmHg SBP, DBP, diabetic complications) variables, as well as sociodemographic parameters (gender, age and diabetes duration in years). PP was calculated as SBP-DBP and MAP as DBP+1/3 PP. Descriptives are given as mean±SD and %. Pearson correlation coefficient was calculated for parametric- and Spearman for non-parametric variables.
**Results:** Results: Data of 622 diabetic patients (197 males, 426 females (68.4%); age 60.24 ± 12.48 years (range 7.1–101.07); 596 (95.7%) type 2 diabetic, 27 (4.2%) type 1 diabetic patients; diabetes duration 13.01 ± 7.4 years (range 2–43.04), with a mean follow-up duration of 2.9 ± 2.3 years (range 0–7.55), a mean control interval of 3.21 ± 2.98 months (range 0–28.35) and a mean control frequency of 12.53 ± 9.95 visits (range 1–31 ) were included in the study. There was no difference between mean $baseline_0$ and $endpoint_x$ metabolic and hemodynamic values as demonstrated by 95% confidence intervals (CI) in table 1. The difference between $baseline_0$ and $endpoint_x$ retinopathy ratio was significant (15.6% vs. 33.0%, difference in proportions 0.17, 95% CI 0.13 to 0.22). The risk ratio (RR) for developing retinopathy over the mean follow up period of 2.9 years for all was 0.57. $Retinopathy_x$ was significantly associated with $PP_x$ ($r = 0.161$, $p < 0.001$), $A1c_x$ ($r=0.158$, $p<0.001$), age ($r= 0.16$, $p<0.001$), diabetes duration ($r=0.308$, $p<0.001$) and mean arterial $pressure_x$ (MAP) ($r=0.112$, $p=0.006$). RR for developing diabetic retinopathy over the mean follow-up period for diabetic individuals with a $PP_0 \leq 40$ mmHg was 1.08 vs. 1.31 for individuals with a $PP0 \geq 50$ mmHg.
**Conclusion:** Although, metabolic and clinical parameters showed no significant change over the mean follow-up period, there was a significant increase in risk of diabetic retinopathy. Baseline PP over 50 mmHg seemed to be indicative of endpoint retinopathy, with a critical span between 40–50 mmHg.

Table 1: Baseline vs. endpoint metabolic and hemodynamic parameters and their differences with 95%CI

| | Mean baseline $value_0$ (range) | Mean endpoint $value_x$ (range) | Mean difference between samples | 95% CI |
|---|---|---|---|---|
| UAER, mg/day | 86.12 ± 391.41 (0 – 5920) | 82.03 ± 358.39 (0.50 – 5950) | 4.09 ± 61.43 | –116.65 to 124.83 |
| A1 c, % | 7.88 ± 2.90 % (2.60 – 37.10) | 7.66 ± 2.39 % (3.10–37.10) | 0.16 ± 0.16 | –0.16 to 0.48 |
| PP, mmHg | 58.02 ± 18.15 (10–130) | 59.42 ± 18.23 (10 – 140) | 1.40 ± 1.06 | –3.47 to 0.68 |
| MAP, mmHg | 103.74 ± 15.49 (63.33 – 166.67) | 103.22 ± 15.10 (63.33 – 166.67) | 0.51 ± 0.89 | –1.24 to 2.26 |
| SBP, mmHg | 142.39 ± 24.35 | 142.86 ± 24.26 | 0.47 ± 1.41 | –3.24 to 2.30 |
| DBP, mmHg | 84.39 ± 13.11 (50 – 150) | 83.44 ± 13.36 (40 – 140 ) | 0.95 ± 0.77 | –0.59 to 2.46 |

## 1060

**Diabetic macular edema – relationship of visual acuity to involvement of the center of the macula**

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**Background and aims:** Vision loss from diabetic macular edema (DME) usually occurs when retinal thickening involves the center of the macula (COM). However, there are few data available concerning the effect of distance from COM in relation to vision loss or progression of DME. The aim of this analysis was to examine the relationship between proximity of retinal thickening to COM and visual acuity (VA) in patients assigned to placebo in two clinical trials.
**Materials and methods:** The PKC-DRS and PKC-DMES trials enrolled 252 & 686 patients, respectively, with nonproliferative diabetic retinopathy and various levels of severity of DME and assigned one-fourth of them to placebo. DME severity was assessed by reading center grading of ETDRS 7-field stereoscopic photographs. The distance from COM (in 100 micron [μ] increments) of the most posterior edge of retinal thickening (or adjacent hard exudate) was estimated.
**Results:** Patients in the PKC-DRS trial had levels of DME severity that ranged from none to center involvement at baseline and 26% of these patients experienced sustained moderate visual loss (i.e., a ≥15 letter loss on the ETDRS chart occurring on 2 consecutive visits 6 mo apart) in the primary study eye. Patients in the PKC-DMES trial had DME >300 to 3000μ from COM at baseline and less than 5% of these individuals experienced sustained moderate visual loss. In the PKC-DRS trial, mean VA at all follow-up visits in all eyes was 82 letters when retinal thickening was >500μ from COM. When retinal thickening was ≤500μ but >300μ from COM, VA was 81 letters; when thickening was ≤300μ without COM involvement, VA was 80 letters, and when COM was involved VA was 60 letters ($p<0.001$ vs. >500μ group). In the PKC-DMES trial, 31% of the 137 eyes with retinal thickening >500μ from COM at baseline eventually developed center thickening. In contrast, of the 46 eyes with retinal thickening >300 but ≤500μ from COM at baseline, 44% ($p=0.149$ vs. >500μ group) eventually developed DME at COM.
**Conclusion:** These data suggest that the closer DME gets to the center of the macula, the more likely it is to eventually involve the center and impair vision.

## 1061

**Retinopathy in subjects with impaired fasting glucose. The NANSY-eye study**

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**Background and aims** Retinopathy is a serious and common complication to diabetes. It may even be seen already at diagnosis of diabetes. It is not known how prevalent retinopathy is in subjects with impaired fasting glucose (IFG), or if it can be prevented by treatment with sulphonylurea in these subjects. The NANSY study is a 5- year placebo-controlled intervention with sulphonylurea investigating if conversion to manifest type 2 diabetes can be prevented. The NANSY-eye study is a subprotocol examining a sample of these patients with fundus photos at baseline after 2.5 and 5 years.
**Materials and methods:** Subjects were surveyed in primary care with repeated fasting blood glucose measurements. Those with two consecutive values ≥ 5.6 and <6.1 mmol $L^{-1}$ were invited to participate. Baseline physical examination included blood pressure in supine position after 5 minutes rest, BMI, and WHR. Venous blood samples have been frozen for later analyses.
The fundus photos was taken after mydratic drops; 35 mm diafilm was used and of each eye photos were taken in two fields. The alternative classification of the Wisconsin Epidemiologic Study of Diabetic Retinopathy was used to classify the retinopathy level.
Hypertension was defined as blood pressure ≥160/90 mmHg (one or both), or ongoing therapy for hypertension.
**Results:** In all, 90 men and 64 women with IFG were surveyed at baseline and examined with fundus photos within six months. Of these, 16 subjects (10%) had signs of retinopathy. There was no difference in occurrence of retinopathy between subjects with known diagnosis of hypertension or not. However, subjects with retinopathy had higher systolic (153 vs 141 mmHg, 95% CI 3–20 mmHg) and diastolic blood pressure (86 vs 81 mmHg, 95% CI 1–10 mmHg) adjusting for differences in age, sex and known hypertension. There was a corresponding difference in BMI, 32.4 vs 29.2 kg $m^{-2}$. There were no associations between fasting blood glucose and retinopathy.
**Conclusion:** Retinopathy is common even before type 2 diabetes is manifest. It is associated with high blood pressure and overweight in a pattern resembling the metabolic syndrome. Further analyses will reveal if treatment with sulphonylurea prevents development of retinopathy in these subjects.

## 1062

**Effects of pancreas transplantation on diabetic retinopathy**

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**Background and aims:** The effects of pancreas transplantation (PTx) on diabetic retinopathy (DR) are still unclear.
**Materials and methods:** We studied the course of DR in 53 patients (age: 38 ± 8 years; males/females 29/24, BMI: 23.2 ± 2.2 Kg/$m^2$, duration of diabetes: 24 ± 8 years) bearing a successful PTx (combined with a kidney in 42 patients, as solitary graft in 11 patients). All patients were examined with indirect and direct retinoscopy, two non-stereoscopic 45° retinal photographs for each eye, and corrected visual acuity. Follow-up ranged 6 to 60 months (median: 15 months). Before transplantation, according to the Eurodiab Study classification, 27 patients (51%) had non-proliferative retinopathy (NPDR, mild, moderate or severe), and 26 patients (49%) had proliferative retinopathy (PDR). During the follow-up, in the non-proliferative group improvement/deterioration was defined as regression/progression to a lower/higher retinopathy grade; in the proliferative and/or laser treated group, stabilization was defined as no new neo-vessel formation or development of other new lesions requiring laser treatment.
**Results:** In the non-proliferative diabetic retinopathy group, 9 (32.1%) eyes of 5 (31.3%) patients improved of 1 or more lesion grading; 17 (60.7%) eyes of 8 (50%) patients showed no change; and 3 (10.7%) eyes of 3 (18.7%) patients progressed of 1 grade. In the proliferative and/or laser treated diabetic retinopathy group, stabilization occurred in 67 (95.7%) eyes of 35 (94.6%) patients; worsening was observed in 3 (4.4%) eyes of 2 (5.4%) patients. A 25% or more reduction of hessudates and/or hemorrhages was found in 49 (70%) of eyes with pre-transplant proliferative and/or laser treated retinopathy.
**Conclusion:** In conclusion, despite a relatively short follow-up period, successful PTx in our cohort of patients improved or stabilized advanced diabetic retinopathy in the vast majority of recipients.

# PS 105

## Experimental retinopathy

### 1063

**Neuro-glial dysfunction in retinae of (mREN-2)27 rats during diabetes**

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**Background and aims:** Recent studies have implicated the renin-angiotensin system in vascular changes during diabetes. In addition to vascular abnormalities, there may be alterations in neuronal and glial function. The aim of this project was to characterize the vascular and neuroglial changes in this animal model.
**Materials and methods:** Sprague-Dawley and hypertensive (mRen-2)27 transgenic rats were rendered diabetic by an intravenous injection of streptozotocin (50 mg/kg). Control rats received injections of citrate buffer alone. Following 4, 10 or 20 weeks of diabetes, retinal function was assessed using the electroretinogram (ERG). In addition, retinal vasculature and neurochemistry was evaluated. Acellular capillaries were examined following digestion of the neural retina with trypsin. Retinal vascular leakage was estimated using the Evan's blue technique. Amino acid immunocytochemistry was performed using antisera directed against glutamate, GABA, glycine, aspartate, alanine, glutamine, arginine, taurine, ornithine and glutamine synthetase (kindly donated by Dr Robert Marc). Changes in subpopulations of neurons and Müller cells were analysed by image analysis using NIH Image.
**Results:** Vascular abnormalties were observed following 10 weeks of diabetes; at this time there was an increase in acellular capillaries in the mid and peripheral retina, which became more marked at 20 weeks (periphery non-diabetic Ren-2, $0.70 \pm 0.007$; periphery diabetic Ren-2, $1.91 \pm 0.44$). This was accompanied by venous beading and microaneurysms. In all groups, vascular leakage was unchanged with diabetes at 4 weeks. By 10 weeks of diabetes, retinal vascular leakage had increased by approximately 2 fold in Ren-2 rats (non-diabetic Ren-2, $6.07 \pm 0.89$; diabetic Ren-2, $13.06 \pm 1.44$ ul plasma g-1 retina weight hr-1). Significant changes in amino acid neurochemistry were observed in the diabetic (mRen-2)27 rat. In particular Müller cells displayed an increase in GABA and arginine immunoreactivity and no change in glutamate or glutamine. There were substantial increases in labelling to aspartate, alanine and ornithine in subpopulations of amacrine cells in the diabetic (mRen-2) 27 rat compared with controls. Retinal function as assessed by the ERG was significantly altered by in diabetic animals.
**Conclusion:** These data suggest that the (mRen-2)27 rat develops marked abnormalities in their vasculature that is accompanied by neurochemical changes in the retina.
*Supported by: NH&MRC #2909974 & #208950; JDRF*

### 1064

**Selective elevation of matrix metalloproteinase-2 in aqueous humour in subjects with proliferative diabetic retinopathy**

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**Background and aims:** Matrix metalloproteinases – zinc containing enzymes that remodel extracellular matrix – have been implicated in various pathological processes including neovascularisation, inflammatory response and cardiovascular disease. Recent data suggest that matrix metalloproteinases might be also implicated in the pathogenesis of diabetic retinopathy, but the exact type of metalloproteinase involved in this process remains yet to be determined.
**Materials and methods:** We measured serum concentrations of MMP-2 and MMP-9, by standard ELISA assays, in 34 patients with diabetes ( 15 with various stages of retinopathy ) aged ( mean +/–SD) 70.2+/–8.1, duration of diabetes 14.97+/–10.2 years. As 14 of those patients also required cataract surgery we measured MMP-2 and MMP-9 in aqueous humour collected during cataract extraction.
**Results:** There was no difference in age between patients with and without diabetic retinopathy (68.2+/–8.6 years *versus* 71.7+/–7.6 years, p=0.21). Duration of diabetes was, however, longer in patients with diabetic retinopathy (20.1+/–8.2 years) in comparison to controls (11.0+/– 10.1 years, p=0.007).There was no difference in serum levels of MMP-2 and MMP-9 in patients with and without diabetic retinopathy (1182+/–88 ng/ml *versus* 1191+/–119 ng/ml, p=0.69; 651+/–250 ng/ml *versus* 609+/–408 ng/ml, p=0.52, for MMP-2 and MMP-9, respectively).
MMP-2 levels in the aqueous humour were much lower than in the serum (98+/–60 ng/ml versus 1270+/–66 ng/ml, p<0.0001), but there was no correlation between the serum and the aqueous levels of MMP-2 (r=–0.12, p=0.67). In aqueous humour, however, we observed approximately 3 fold elevation of MMP-2 (177+/–41 ng/ml) in patients with proliferative diabetic retinopathy (n=5) in comparison to patients either without retinopathy, or with the background changes only (66+/–27 ng/ml, p=0.00003). In contrast to MMP-2, the levels of MMP-9 were undetectable by standard ELISA assay in all but two patients with severe proliferative diabetic retinopathy.
**Conclusions:** To the best of our knowledge we demonstrated for the first time preferential activation of matrix metalloproteinase-2 in subjects with proliferative diabetic retinopathy. It remains to be established whether inhibition of matrix metalloproteinase system may play a role in prevention and/or treatment of diabetic retinopathy.
*Partially supported by an EU grant*

### 1065

**Interleukin-1 beta and diabetic retinopathy**

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**Background and aims:** Oxidative stress is increased in the retina in diabetes, and long-term administration of multi-antioxidants to diabetic rats inhibits the activation of apoptosis execution enzyme in the retina and the development of retinopathy. Diabetic retinopathy is shown to have several characteristics of a chronic inflammatory disease. Further, increased oxidative stress, cytokine production and microvascular disorders are considered to be correlated. The purpose of this study is to investigate proinflamatory cytokine, IL-1beta (IL-1B), in the retina in diabetes, and its relation to increased oxidative stress.
**Materials and methods:** A group of alloxan diabetic rats received diet supplemented with multi- antioxidants (Trolox, alpha tocopherol, N-acetyl cysteine, ascorbic acid, beta-carotene and selenium) for up to eight months. Oxidative stress, NO, and IL-1B expression (western blot) and its content (ELISA) were measured in the retina at two and eight months of diabetes.
**Results:** Two months of diabetes increased IL-1B content in the retina by 30%, and IL-1B remained elevated at eight months of diabetes. Similarly, the protein expression of IL-1B was elevated by 40% in the retina obtained from diabetic rats. In the same retina, both lipid peroxides and NO were elevated by over 50% compared with age-matched normal rats. The duration of diabetes had no effect on IL-1B content or its protein expression. Administration of multi-antioxidants inhibited diabetes-induced elevations in retinal IL-1B (both the levels and protein expression), oxidative stress and NO without ameliorating the severity of hyperglycemia. Similar beneficial effects were observed when diabetic rats were administered alpha lipoic acid (400 mg/kg) instead of multi-antioxidants.
**Conclusion:** These results show that IL-1B is elevated in retina in diabetes before either cell death or histopathology can be seen, and remains elevated for the duration of diabetes when capillary cell death and retinopathy are developing. Antioxidants might be inhibiting the development of retinopathy by inhibiting accumulation of the cytokine.
*Supported by: Juvenile Diabetes Research Foundation*

### 1066

**Troglitazone reverses the inhibition of nitric oxide production by high glucose in cultured bovine retinal pericytes**

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**Background and aims:** In the retinal microcirculation, there is a basal release of nitric oxide (NO) which maintains the retinal blood flow in a constant state of vasodilation. The ratio of endothelial cells to pericytes in the retinal capillaries is 1:1 and pericytes may be important in the regulation of microcirculatory hemodynamics in the retina. It has been suggested that the pathogenesis of diabetic retinopathy may involve a reduced

bioavailability or diminishing production of NO. The aim of the present study was to elucidate the effect of high glucose on NO synthesis and to test the effect of troglitazone, a potent agonist of peroxisome proliferator activated receptor-gamma (PPARγ), on NO production by bovine retinal pericytes (BRP).
**Materials and methods:** Primary cultured BRP were grown in normal (5.5 mM) or high (22 or 30 mM) glucose medium for 48 hours. NO production by BRP was measured as nitrite (a stable metabolite of NO) concentration in cell-culture supernatants. The inducible NOS (iNOS), which is responsible for NO production in BRP, was studied by immunoblot. Intracellular superoxide production was measured by flow cytometry.
**Results:** A dose-dependent reduction of NO release occurred 48 hours after exposure to 22 or 30 mM glucose with concomitant reduction in iNOS expression. Troglitazone increased NO release and iNOS expression in a time- and dose-dependent manner with no alteration in ROS production. The maximal increase (~1.8 fold) was achieved with 20 uM troglitazone treatment for 12 hours. Furthermore, bisphenol a diglycidyl ether (BADGE) (5 uM), a PPARγ agonist, inhibited (~60%) troglitazone-stimulated NO production and these data support the increase of NO release is the consequence of an effect of troglitazone as a PPARγ agonist. Addition of troglitazone (20 uM) to media reversed the inhibitory effect of high glucose on BRP NO production.
**Conclusions:** Our data suggest that high glucose compromises NO release in BRP and PPARγ agonist restore diminishing production of NO.

## 1067

**Bovine retinal pericytes seem resistant to glucose-induced oxidative stress in vitro**

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**Background and aims:** Diabetic retinopathy is a sight-threatening long-term complication to diabetes, and loss of pericytes toghether with basement membrane thickening represent early signs of its development. The pathogenesis of diabetic retinopathy is complex and not yet fully understood. Recent studies have suggested that oxidative stress induced by hyperglycemia may play a role. In the present study we tested the hypothesis that high glucose may induce signs of oxidative stress in bovine cultured pericytes.
**Materials and methods:** Pericytes were exposed to either normal (5.5 mM) or high (22 mM) glucose levels for 1, 3 and 5 days. Signs of oxidative stress were measured by the mRNA expression of the anti-oxidant enzymes MnSOD, catalase and glutathione peroxidase using real-time PCR. Furthermore, in order to elucidate the role of oxidative stress we also measured glutathione (GSH) concentration in the cells and investigated the impact of thiol reactive metal ions and hydrogen peroxide on intracellular GSH incubated in high and normal glucose environment.
**Results:** Despitethe stimulation with high glucose, thiol reactive metal ions or hydrogen peroxide, there was no clear increased expression of anti-oxidant enzymes or influence of GSH levels.
**Conclusion:** These data indicate, that under the conditions used, pericytes do not develop oxidative stress in response to hyperglycemia. Thus, oxidative stress does not seem to be a major cause of pericyte loss in the development of diabetic retinopathy.

## 1068

**Interleukin-8, monocyte chemoattractant protein-1 and interleukin-10 in the vitreous fluid of patients with proliferative diabetic retinopathy**

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**Background and aim:** Recent research has provide growing evidence indicating that inflammation is an important event in the etiopathogenesis of diabetic retinopathy. However, it could be postulated that the balance between pro-inflammatory and anti-inflammatory cytokines rather than proinflammatory cytocines alone would be a primary factor in determining the development of PDR. On this basis, the aim of the study was to determine the intravitreous levels of two pro-inflammatory cytokines (IL-8, MCP-1) and the anti-inflammatory cytokine IL-10 in patients with proliferative diabetic retinopathy (PDR). In addition, the relationship between the profile of cytokines and PDR activity has also been evaluated.
**Material and methods:** The study included 22 consecutive diabetic patients with PDR (6 type 1 and 16 type 2) on whom a vitrectomy was performed. Sixteen age-matched non-diabetic patients with other conditions requiring vitrectomy, but in which the retina was not directly afected by neovascularization served as a control group. IL-8, MCP-1 and IL-10 were measured by ELISA.
**Results:** The vitreal levels of both IL-8 and MCP-1 were strikingly higher in diabetic patients with PDR in comparison with the control group (173.5 pg/ml [64–1670] vs. 49 pg/ml [25–145], p<0.001, and 2.171 pg/ml [388–6155] vs. 438 pg/ml [207–1344], p<0.001; respectively). In addition, the vitreous concentrations of IL-8 and MCP-1 were higher in patients with active PDR than in those patients with quiescent PDR (324.5 pg/ml [80–1670] vs. 173.5 pg/ml [64–487], p=0.06 and 3.596 pg/ml [1670–6155] vs. 1.1433 [388–2500], p=0.01; respectively). However, vitreal levels of IL-10 in diabetic patients were similar to that obtained in the control group (2,89 pg/ml [1,55–5,50] vs. 2,46 pg/ml [2,2–5,41], p=ns).
**Conclusion:** Our results suggest that IL-10 does not counterregulate the proinflammatory and angiogenic effect of IL-8 and MCP-1 in diabetic patients with PDR.
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## 1069

**Thiamine and benfotiamine inhibit the polyol pathway and increase transketolase mRNA in endothelial cells and retinal pericytes cultured in high glucose**

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**Background and Aims:** Hyperglycemia is the major causal factor for the development of the vascular complications of diabetes but the mechanisms have not been fully elucidated. Biochemical mechanisms that could be involved include increased glucose flux through the polyol pathway, enhanced nonenzymatic protein glycation, accelerated generation of reactive oxygen species (ROS), and activation of the diacylglycerol-protein kinase C (PKC) pathway. We and others showed previously that thiamine (T), the coenzyme for transketolase (TK), and benfotiamine (BT), a lipophilic analogue, are able to correct increased lactate production, glycation, ROS formation and PKC activation due to high glucose in endothelial cells. Aldose reductase (AR), the first enzyme of the polyol pathway, trasforms D-glucose into D-sorbitol, leading to imbalances in intracellular homeostasis. The aim of this study was to verify if T and BT modify AR mRNA expression and activity, along with sorbitol concentrations, in human umbilical vein endothelial cells (HUVEC) and bovine retinal pericytes (BRP) cultured in high glucose. TK mRNA expression was also investigated in the same cells.
**Material and methods:** HUVEC and BRP were cultured for 7 days in normal (5.6 mmol/l, G5.6) or high (28 mmol/l, G28) glucose concentrations, with or without T 50 and 100 μmol/l or BT 50 and 100 μmol/l. AR and TK mRNA expression was determined by relative quantitative RT-PCR and the results expressed as the ratio over beta-actin, taken as house-keeping gene. AR activity was determined spectrophotometrically by measuring the decrease of NADPH. Sorbitol concentration was assayed by GC-MS. Results are means ± SD of 6 experiments, expressed as percentages of values in G5.6, except for AR activity, expressed as U/mg protein.
**Results:** Both T and BT, when added to high glucose, reduced significantly AR mRNA expression and activity. Sorbitol concentrations were increased in high glucose in both HUVEC and BRP and reduced by T and BT. TK mRNA was increased by T e BT only in the presence of high glucose in both HUVEC and BRP.

*p<0.05 vs. G5.6; **p<0.05 vs. G28

| HUVEC | G5.6 | G28 | G28T50 | G28T100 | G28BT50 | G28BT100 |
|---|---|---|---|---|---|---|
| AR mRNA | 100 | 183.5 ± 36.3* | 162.0 ± 42.3** | 139.8 ± 29.0** | 158.7 ± 35.5** | 141.7 ± 21.5** |
| AR Activity | 1.8 ± 0.6 | 6.5 ± 0.9* | 3.7 ± 0.6** | 3.4 ± 0.6** | 3.8 ± 1.6** | 4.0 ± 1.2** |
| Sorbitol | 100 | 177.9 ± 52.1* | 140.5 ± 30.8 | 124.0 ± 14.1** | 161.3 ± 36.1 | 143.6 ± 31.1 |
| TK mRNA | 100 | 92.7 ± 10.5 | 141.5 ± 23.3** | 167.6 ± 27.9** | 127.9 ± 10.2** | 171.1 ± 21.6** |
| **BRP** | **G5.6** | **G28** | **G28T50** | **G28T100** | **G28BT50** | **G28BT100** |
| AR mRNA | 100 | 218.9 ± 41.6* | 162.9 ± 41.6** | 143.5 ± 33.5** | 160.7 ± 43.2** | 137.9 ± 36.2** |
| AR Activity | 1.1 ± 0.2 | 4.5 ± 0.3* | 2.6 ± 0.3** | 2.7 ± 0.6** | 2.9 ± 0.4** | 2.7 ± 0.6** |
| Sorbitol | 100 | 346.2 ± 52.2* | 277.8 ± 71.2 | 266.4 ± 78.1 | 288.0 ± 83.6 | 272.1 ± 64.5 |
| TK mRNA | 100 | 109.4 ± 25.3 | 202.9 ± 57.2** | 217.3 ± 69.1** | 180.4 ± 52.8** | 218.6 ± 66.3** |

**Conclusions:** T and BT reduce polyol pathway activity and enhance TK mRNA expression in vascular cells cultured in high glucose. These and other positive effects observed in high glucose, suggest that test this vitamin may have the potential to reduce vascular damage in diabetes.

# PS 106

# Nephropathy – epidemiology/intervention

## 1070

**Burden of diabetic nephropathy in a comprehensive diabetes care centre in Bangladesh**

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**Background and aims:** Diabetic nephropathy (DN) is one of the main causes of morbidity and mortality all over the world and it creates special health and socioeconomic burden in developing countries due to lack of organized health care services. Designing a proper intervention policy for the problem requires an understanding of the extent of the problem. This study was undertaken, for the first time, to explore the prevalence and cost burden of DN in Bangladesh in a comprehensive care hospital.
**Materials and methods:** Prevalence of DN was determined by retrospective cohort study of patients with diabetes from January 1988 to December 2003 in the Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), the central institute of the Diabetic Association of Bangladesh (DAB), providing comprehensive diabetic care to a large number of diabetic patients (total number of registered patients are 0.23 million up to February, 2004). Sixty patients with DN attending the Nephrology Department, BIRDEM were selected randomly and they were interviewed in the month of February 2004 with a preset questionnaire along with scrutinization of guide book records regarding the direct cost (cost of medical advice, investigations, medical and surgical treatment) and indirect cost [travel cost, cost of productivity loss, and cost of accompanying person(s)]. The outcome measures were life expectancy (in life-years [LY]) and quality adjusted life expectancy (in quality-adjusted life-years [QALY]). A comparison was made between patients undergone invasive management (Group 2, n=30) and others (Group 1, n=30) and it was expressed as cost-effectiveness ratio.
**Results:** Among 905 patients identified with diabetes 197 (21.8%) were found to develop diabetic nephropathy. The total cost of management was US$ 49,034.20. with an average of US$ 817.24. The cost of Group 2 was 13.4 times greater than that of Group 1. Group 2 was associated with 10.30 LY and 11.18 QALY whereas Group 1 was associated with 23.30 LY and 31.18 QALY. From the point of simple mathematical calculation, Group 1 was 19.2 times lower in cost per QALY than Group 2, i.e. timely intervention was found to be significantly cost-effective. For Group 2, the value of odds ratio was 10.91 and it showed that Group 2 patients had to spend 10.91 times more for those with severity and complication level of nephropathy in this group ($\chi^2$= 13.47, df= 1, p= 0.01).
**Conclusion:** The cost of DN is high in a developing country even when calculated in a social welfare organization without profit motive (i.e. at a reasonable BIRDEM rate). A substantial burden of care of DN in Bangladesh is in the form of invasive management.The huge burden despite comprehensive care is only the tip of iceberg and needs resource mobilization and careful health planning.
*Supported by: Diabetic Association of Bangladesh*

## 1071

**Prevalence of chronic diabetic complications in aging patients according to body mass index**

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**Background and aims:** Type 2 diabetes (DM2) is a major risk factor for cardiovascular disease (CVD) and its prevalence increases with aging. Obesity represents a risk factor for DM2, CVD and global mortality.
The aim of this study was to evaluate the effect of body mass index (BMI) on chronic diabetic complications in aging DM2 patients
**Materials and methods:** 4465 consecutive DM2 patients, aged > 45 years. Patients were classified according their BMI (31 kg/m$^2$) and age (45–64, 65–74, 75–85 and >85 years). Statistical analysis was done using ANOVA test was performed for quantitative and Chi-squared for qualitative data.
**Results:** Years of diabetes evolution increased with age except for the oldest group. The oldest and the youngest had similar duration of diabetes. The lowest BMI was found in the oldest group (p<0.0001). We did not find any

difference across age groups in HbA1 c, total-cholesterol or HDL-cholesterol. The oldest group had significantly lower triglycerides ($p<0.05$), % hypertension, % smoking and % sedentary. Prevalence of diabetic chronic complications war lower in the youngest group, and it increased with age. The highest prevalence for cerebrovascular disease ($p<0.0003$), polyneuropathy ($p<0.00001$) and diabetic foot ($p<0.00001$) was found in the oldest group. Retinopathy ( $p<0.001$) and nephropathy ($p<0.001$) were more prevalent in the youngest group. The relation between BMI and chronic complications was different between groups of age. In the youngest group a higher BMI was related to a higher prevalence of ischemic heart disease ($p<0.0002$) and nephropathy ($p<0.0009$) and a lower prevalence of peripheral angiopathy ($p<0.01$) and retinopathy ($p<0.0001$). In the oldest group a greater BMI was associated with a lower BMI was related to a lower prevalence of cerebrovascular disease ($p<0.00001$), peripheral angiopathy ($p<0.0006$), diabetic foot ($p<0.0009$) and nephropathy ($p<0.03$). In the oldest group an increase of BMI was not associated with an increase of ischemic heart disease.
**Conclusion:** Overweight in DM2 is associated with a higher prevalence of chronic complications between ages 45–65 years and with a lower in ages >75 years. Prospective studies would help us to evaluate the effect of body weight on chronic diabetic complications in aging diabetic patients.

## 1072

**Topiramate decreases weight and improves HbA1c and urinary albumin excretion in obese subjects with Type 2 diabetes**

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**Background and aims:** Progression of urinary albumin excretion (UAE) is predictive of cardiovascular morbidity and mortality in patients with diabetes. Poorer glycemic control in patients with diabetes over time correlates with development and progression of UAE. Topiramate (TPM) shown to promote weight loss, is known to be beneficial in obese patients with type 2 diabetes (DM2). This study compared the efficacy and safety of TPM vs placebo (PBO) in obese subjects with recently diagnosed DM2 naïve to treatment.
**Materials and methods:** Obese patients (18–75 y, $27 \leq BMI < 50\ kg/m^2$) newly diagnosed with DM2 were enrolled in a 6-wk PBO run-in. During run-in, nonpharmacologic therapy (Pathways to Change® program) consisting of a 600 kcal deficit diet, behavioral modification program, and physical activity program that continued throughout the trial was started. Subjects with known renal disease, renal insufficiency (serum creatinine >133 µmol/L in males and >124 µmol/L in females) or proteinuria (≥500 mg/d) were excluded. At baseline (BL) (after 6-wk run-in), 541 subjects were randomized to PBO or 96 or 192 mg/d TPM for 60 wks. Sponsor ended study early to develop formulation with enhanced tolerability. 24-hour UAE was measured at BL and wk 32.
**Results:** Subjects in both TPM groups with both BL and 32-week evaluations had statistically significant weight loss and reductions in HbA1 c, systolic (S) and diastolic (D) blood pressure (BP), and UAE from BL to wk 32 vs PBO (Table). Most common adverse events that occurred over trial duration for all randomized subjects and in ≥5% of TPM subjects and >PBO were CNS-related and included paresthesia, fatigue, hypoesthesia, concentration difficulty, memory difficulty, and vertigo.
**Conclusion:** In obese subjects with DM2 naïve to treatment and without renal disease at baseline, TPM decreased body weight, HbA1 c, SBP, DBP, and UAE.

Change from baseline to week 32, observed population

| Mean values | PBO (n = 110) | TPM 96 mg/d (n = 92) | TPM 192 mg/d (n = 99) |
|---|---|---|---|
| Baseline BMI ($kg/m^2$) | 36.3 | 36.7 | 35.8 |
| Baseline body weight (kg) | 104.2 | 107.6 | 104.1 |
| Change in body weight at 32 weeks (%) | −3.2 | −8.0* | −8.6* |
| Baseline HbA1 c (%) | 6.6 | 6.8 | 6.7 |
| Change in HbA1 c at 32 weeks (%) | −0.2 | −0.6* | −0.6* |
| Baseline SBP/DBP (mmHg) | 132.8/80.1 | 133.7/80.0 | 133.0/80.9 |
| Change in SBP/DBP at 32 weeks(mmHg) | −2.0/0.4 | −6.1**/−3.0* | −9.1*/−4.1* |
| Baseline UAE (mg/24 h) | 19.6 | 29.9 | 30.0 |
| Change in UAE at 32 weeks (mg/24 h) | 0.5 | −17.6* | −13.9** |

*$P \leq 0.001$ vs PBO; **$P \leq 0.009$ vs PBO.

## 1073

**Efficacy of fosfomycin in the treatment of recurrent uncomplicated lower urinary tract infections in Type 2 diabetes mellitus**

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**Background and aims:** Recurrent lower urinary tract infections (UTIs) are a major health problem for diabetes patients, resulting in frequent office visits and often requiring the use of antimicrobial agents. Fosfomycin seems to be a very interesting alternative for the treatment of UTIs. However, there is not clinical data on the efficacy of this agent in the long-term treatment. The aim of this study was to evaluate the efficacy of fosfomycin in treatment and prevention of UTIs in type 2 diabetes women.
**Materials and methods:** The study comprised 100 type 2 diabetes women (mean age 60.1 ± 7.2 years, duration of diabetes 10.7 ± 4.2 years, glycated hemoglobin A1 c 5.4% ± 0.6) with clinical signs of UTIs and positive urine culture (isolated bacterial uropathogen susceptible to fosfomycin and/or nitrofurantoin), with often UTIs in the medical history (at least 4 episodes of UTIs within the last 18 months). The women were divided into two similarly numerical randomized groups depending on the applied treatment. Group I has been applied orally fosfomycin 3g at 22.00 p.m. every 30 days for the next 6 months. The control group II was applied orally nitrofurantoin 0.1g every 12 hours before meals for 7 days, and then 0.1 g. every day for the next 6 months. The patients remained under regular medical supervision for 12 months in outpatient clinic in Diabetology Department of the Medical University of Lodz. Efficacy of these agents were assessed on the ground of urinalysis and urine culture performed every 3 months for 12 months during clinic visits in Diabetology Department.
The results were analyzed according to well known statistical methods by using StatSoft Statistica for Windows, release 6.0 (StatSoft, Inc., Tulsa, USA). To determined difference between and within groups two-sided test for proportions was used. P-values < 0.05 were considered to be significant.
**Results:** After six months of long-term treatment with fosfomycin in about 4/5 of patients the therapeutic success (eradication of uropathogen and disappearance of clinical symptoms of UTIs) was observed. After twelve months (six months after discontinuation of fosfomycin-therapy) in above 3/4 of patients therapeutic success was observed. There was no significant difference in the efficacy of treatment between fosfomycin (F) and nitrofurantoin (N) during long-term therapy: after 3 months (F vs. N, p=0,122) and after 6 months (F vs. N, p=0,122). There was significant difference in a frequency of recurrence of UTIs in subjects, depending on the antimicrobial agent applied before. In comparison with control group II, in group I rare episodes of UTIs both in the $9^{th}$ month (F vs. N, p=0,025) and in the $12^{th}$ month of study (F vs. N, p=0,023) were observed.
**Conclusion:** Fosfomycin is an effective agent both in the treatment and prevention of UTIs. It can be a recommended antimicrobial agent in chronic treatment of UTIs in type 2 diabetes patients.

## 1074

**Resistance of E. coli in hospitalized diabetic patients**

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**Background and aims:** E coli is a common pathogen of urinary tract infections (UTIs) whose resistance in several antibiotics increases steadily. The aim of this study was to investigate antibiotic resistance of E.coli isolated from patients hospitalized with urinary tract infections and to compare susceptibilities between diabetics and non-diabetics.
**Materials and methods:** 680 patients (64% females) with UTIs, hospitalized during a two-year- period, were studied. Mean age of our patients was 68.9 years (median 76, range 6–93).
**Results:** 74 patients were diabetics, 63 females and 11 males. E coli was isolated from 354 patients (52.0%), 275 (77.7%) females and 79 (22.3%) males. Resistance of E coli in antibiotics is shown in Table 1.

TABLE 1

| ANTIBIOTIC | DIABETICS | NON-DIABETICS | |
|---|---|---|---|
| AMPICILLIN | 39 **52.7%** | 112 **40.0%** | **p<0.05** |
| COTRIMOXAZOL | 25 **33.8%** | 76 **27.1%** | **NS** |
| AMOX/CLAV | 8 **10.8%** | 25 **8.9%** | **NS** |
| PIPERACILLIN | 12 **16.2%** | 48 **17.1%** | **NS** |
| GENTAMYCIN | 6 **8.1%** | 5 **1.8%** | **p<0.05** |
| AMICACIN | 2 **2.7%** | 0 **0.0%** | **P<0.05** |
| CIPROFLOXACIN | 2 **2.7%** | 21 **7.5%** | **NS** |

**Conclusion:** Resistance of E coli in ampicillin and aminoglycosides was significantly increased in hospitalized diabetic patients with UTIs comparing with non-diabetics, but differences were not statistically significant (NS) for co-trimoxazole, amoxicillin/clavulanate, piperacillin and quinolones. Increased resistance in diabetics is related probably with increased consumption of antibiotics, more hospitalizations and longer duration of hospitalization.

## 1075

**Nurse-led diabetic renal clinic: does it make a difference?**

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**Background and aims:** Patients with Diabetic Nephropathy (DN) are at a high risk for ESRD and increased CV morbidity and mortality. Strict blood pressure and glycemic control, use of aspirin and statins can ameliorate such risks.
Nurse-led clinics have been shown to improve care outcomes in some chronic circulatory diseases like CCF and CAD and also those with Type 2 diabetes and high blood pressure (BP) as compared to conventional care. There is no current data on specific nurse-led clinic in patients with diabetic nephropathy (DN).
The main objective of this clinic was to see if this additional protocol-based nurse-led clinic could influence cardiovascular risk and halt progression of renal disease in patients with DN.
**Materials and methods:** This was a prospective follow-up of DN patients with specific emphasis on achievement of glycemic control (HbA1 c), target BP and appropriate use of Cholesterol-lowering agents and Aspirin. Patients were seen every 2–3 months (3–4 week interval if needed) and preliminary results analysed over 10 months (March–December 2003). All patients seen at least twice and those with microalbuminuria > 30 mg/mmol (normal < 2.5 mg/mmol) and/or serum creatinine >150 µmol/l (normal 44–133 µmol/l) were included. Information on diabetic kidney disease, diet, exercise, salt restriction alcohol and smoking were given.
BP was measured on 3 occasions 5 min apart after a rest and mean reading taken. Dose titration/additional medication was suggested according to protocol. Letters were sent out to the patients general practitioners (GP) who were advised to check BP monthly and write in the patient diary.
**Results: (see table)**
Total number of patients: 33 (all had Type 2 Diabetes)
- 18 males, 15 females
- 15 Asians, 11 Afro-Caribbeans and 7 Caucasians
- Mean age: 63 years
- Average duration of follow-up: 5 (range 3–10) months
- 25 patients were on Insulin, 8 on oral agents

Urinary ACR was improved in 19/32 (59%) patients by a mean of 50.37 mg/mmol. Serum creatinine was stable in 7/33 (21%), improved in those with abnormal values in 10/33 (30 %) patients. GFR improved in 17/32 (53 %) patients by a mean of 7 ml/min.
88 % patients were on renoprotective agents (18 on ACE inhibitor, 8 on Angiotensin Receptor Blockers and 3 on both). 25/33 (76%) patients were receiving statins (8 patients newly started). 23/33 (70%) were on Aspirin (4 newly started). Average number of BP medication was 3 and in 12/33 patients (36%) one or two BP agents were newly added.
**Conclusion:** There was a positive impact of the nurse-led clinic in this high-risk group of patients even in this short period. Both systolic and diastolic BP was improved significantly and perhaps was the main reason for the positive influence on renal function (creatinine and GFR) and also urinary ACR. Patients are being successfully encouraged to stop smoking. Use of ACE inhibitor/ARB, statins and aspirin is increasing. Glycemic control remains sub-optimal. Hopefully, over a longer period of follow-up, the latter will improve and so should the overall CV risk of these patients with diabetic nephropathy

Nurse - led clinic results (mean+/–SD)

| | Baseline (March 2003) | End study (December 2003) | P value |
|---|---|---|---|
| Mean HbA1 c | 8.6(1.9) | 8.1(1.6) | 0.06 |
| Mean SBP | 155(27) | 139(18) | 0.002 |
| Mean DBP | 85(15) | 79(11) | 0.03 |
| Mean Total Cholesterol | 4.7(1.1) | 4.4(1.1) | 0.16 |
| Mean Triglyceride | 2.1(1.1) | 1.9(0.8) | 0.13 |
| Mean HDL | 1.2(0.4) | 1.2(0.5) | 0.45 |
| Mean weight | 89.7(18.4) | 90.6(19.8) | 0.27 |
| Mean creatinine | 152(8) | 156(7) | 0.36 |
| Mean urinary ACR | 101.6(129) | 105.1(115) | 0.81 |
| Mean GFR | 65.9(37.3) | 63(38.3) | 0.27 |

# PS 107

# Clinical nephropathy: the renin-angiotensin system

## 1076

**Beneficial effect of dual blockade of the renin-angiotensin system on urinary connective tissue growth factor in Type 2 diabetic patients with nephropathy**

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**Background and aims:** Connective Tissue Growth Factor (CTGF) is an important prosclerotic cytokine implicated in the pathogenesis of diabetic glomerulopathy and urinary CTGF (U-CTGF) is significantly increased in patients with diabetic nephropathy. We evaluated short-term changes in U-CTGF of dual blockade of the renin-angiotensin-system (RAS) by adding an angiotensin II receptor blocker (ARB) to treatment with maximal recommended doses of ACE-inhibitor (ACEI) in patients with type 2 diabetes (T2D) and nephropathy
**Materials and methods:**Twenty T2D patients with hypertension and nephropathy were enrolled in this double-blinded randomized two-period crossover trial. Patients received eight weeks therapy with the ARB candesartan16 mg daily and placebo, added in random order to existing treatment with lisinopril/enalapril 40 mg or captopril 150 mg daily. At the end of each treatment period we evaluated: U-CTGF (ELISA, Fibrogen), albuminuria (turbidimetry), 24-h ambulatory blood pressure measurement ((ABPM), Takeda-TM2420) and glomerular filtration rate ((GFR), 51Cr-EDTA plasma-clearance technique)
**Results:** During mono blockade of the RAS by ACEI treatment alone U-CTGF was 5248 (3496 to 7878) ng/24-h (geometric mean (95%CI), albuminuria was 706(349 to 1219) mg/24-h, 24-h ABPM(mean(SEM)) 138(3)/72(2) mm Hg and GFR 77(6) ml/min/1.73 $m^2$. During dual blockade of the RAS by addition of candesartan 16 mg daily, there was an overall mean reduction (95% CI) in U-CTGF of 18 (0 to 33) %, as compared to ACEI alone (p=.05). Albuminuria was reduced by 28(17 to 38) % (p<0.001) and there was a modest reduction in systolic/diastolic 24-h ABPM of 3(-2 to 8)/2(-2 to 5) mm Hg (NS) and in GFR of 4(-1 to 9) ml/min/1.73 $m^2$ (NS).
Interestingly, there was a significant carry-over effect by dual blockade on U-CTGF as reflected by a 36 (17 to 51) % (p<0.001) reduction in those 10 patients who received ACE-I alone in the first period and dual blockade in the second period, whereas there was an insignificant change in U-CTGF of -5 (-38 to 20) % (p=0.71) in patients who received dual blockade in the first period and mono blockade with ACE-I in the second period. A significant carry-over effect was not observed for albuminuria, SBP or GFR.
**Conclusion:** Our short-term study demonstrates that dual blockade of the RAS induces a significant reduction in U-CTGF and albuminuria as compared to monoblockade ot the RAS by ACE-I in maximally recommended doses. A significant carry-over effect on U-CTGF may suggest a prolonged effect of candesartan.
*Supported by: Nierstichting Nederland, Diabetesfonds Nederland*

## 1077

**Beneficial impact on cardiovascular risk factors by dual blockade of the renin-angiotensin system in diabetic nephropathy**

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**Background and aims:** Activation of the renin-angiotensin system (RAS) plays a fundamental role in diabetic nephropathy and blocking the RAS with ACE-inhibitors (ACE-I) is the first line therapy for kidney protection in type 1 diabetic patients. Despite such treatment patients with diabetic nephropathy have a high risk of cardiovascular disease and end stage renal disease. Dual blockade of the RAS with both ACE-I and angiotensin II receptor blockers (ARBs) may offer more complete interruption of the RAS and therefore could improve renal and cardiovascular outcome. We have investigated the short-term effects of dual blockade of the RAS on cardiovascular and renal risk factors in type 1 diabetic patients with diabetic nephropathy compared to ACE-I treatment alone.
**Materials and Methods:** We performed a combined analysis of three randomised, double blind, cross-over trials where 51 patients received 8 weeks of both dual blockade of the RAS using ARBs (irbesartan 300 mg or valsartan 80 mg) in combination with ACE-I (captopril 100 mg or enalapril 20 mg or benazepril 20 mg or enalapril 40 mg) and monotherapy with the same ACE-I. At the end of each treatment period the cardiovascular risk profile were determined.
**Results:** Compared to ACE-I dual blockade of the RAS lowered blood pressure 7/5 mmHg from 137/76 mmHg, decreased albuminuria 37% from 558 mg/24 hour and lowered LDL-cholesterol 0.3 from 3.1 mmol/L (p < 0.01 for all comparisons). The antialbuminuric response to dual blockade of the RAS was influenced by the ACE/ID polymorphism in the gene coding for angiotensin converting enzyme. Patients carrying the D allele had a significant poorer response (mean 31 (95% CI 16 to 43) %) compared to patients with the II genotype (55 (35 to 69) %), p=0.021.
**Conclusion:** Dual blockade of the RAS is a new treatment concept, which may offer additional cardiovascular and renal protection in type 1 diabetic patients with diabetic nephropathy. The response to therapy may be influenced by genetic factors.

## 1078

**The effect of combining angiotensin receptor blocker and angiotensin converting enzyme inhibitor on albuminuria in Type 2 diabetic patients with nephropathy**

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**Background and aims:** Angiotensin converting enzyme inhibitor (ACE-I) and angiotensin receptor blocker (ARB) have been shown to reduce albuminuria and preserve renal function in patients with diabetic nephropathy. Both agents are thought to confer renal protection via blockade of renin-angiotensin-aldosterone system (RAS). Simultaneous blockade of RAS at different levels with ACE-I and ARB may have synergistic anti-albuminuric effect compared to monotherapy.
The study aims to compare the effect of combining Enalapril and Losartan with that of administering either drug alone on 24-hour urine albumin excretion (UAE) in patients with type 2 diabetes mellitus (DM) with nephropathy.
**Materials and methods:** Twenty-eight patients (mean age 57 ± 9.6 years, 7 females) were prospectively studied. After a 4-week washout period, 13 patients received Losartan 100 mg once daily (od) and 15 received Enalapril 20 mg od for 8 weeks. Following this, all 28 patients received a combination of Enalapril 10 mg and Losartan 50 mg od for 8 weeks, followed by a double dose of both drugs for another 8 weeks. Blood pressure (BP), glycosylated hemoglobin and UAE were monitored.
**Results:** Baseline characteristics (age, BMI, duration of DM, HbA1 c, BP, creatinine clearance and UAE) were similar in both groups. There was a significant reduction in mean (95% CI) UAE after 8 weeks of monotherapy [18.0% (4.3% to 31.6%) p=0.002]. The reduction in UAE was significant in patients treated with Losartan [23.9% (2.3% to 45.5%) p=0.01] but failed to reach statistical significance for patients treated with Enalapril [11% (-7% to 29.2%) p= 0.078]. Mean BP reduction was not significantly different between these 2 groups (p= 0.66). After 16 weeks of combination therapy, there was further 11.5% reduction in UAE [(-9% to 32%), p = 0.017] while mean BP and HbA1 c were not statistically different at the begining (week 8) and end (week 24) of the combination therapy.
**Conclusion:** The study showed superior effect of ARB (Losartan 100 mg od) over ACE-I (Enalapril 20 mg od) in reducing albuminuria in patients with diabetic nephropathy. The combination of both drugs showed further benefit in albuminuria reduction independent of BP control.
*Funded by: National Healthcare Group*

## 1079

***ACE2* and *ACE* polymorphisms and nephropathy in Type 1 diabetes**

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**Background and aims:** The renin-angiotensin-aldosterone system (RAAS) is a key regulator of blood pressure and one of the most important contributors to the progression of diabetic nephropathy. Most studies have so far focused on the angiotensin-converting-enzyme (ACE), but its role in the pathogenesis of diabetic nephropathy is still controversial. Recent studies show a complex system with far more components, i.e. *ACE2* gene, that might contribute to the genetic susceptibility to nephropathy in a subset of

diabetic patients. In this respect, it is of note that *ACE2* protein expression has been shown to be reduced in the diabetic kidney in rats. The aim of this study was to evaluate if polymorphisms of the *ACE* and *ACE2* genes are associated with diabetic nephropathy in type 1 diabetes.
**Materials and methods:** We studied 1288 type 1 diabetic patients (460 normoalbuminuric, 276 microalbuminuric, 368 macroalbuminuric and 184 ESRD) from the ongoing FinnDiane Study. In addition to demographic data, blood and urine samples for determination of e. g. $HbA_{1c}$, lipids and AER were collected. Specific information about antihypertensive medication was also obtained. S-ACE was measured by RIA. In addition, 184 non-diabetic healthy blood donors from different parts of Finland were genotyped and used as control subjects. Five evenly distributed SNPs for the *ACE* and four for the *ACE2* were genotyped using TaqMan® technology.
**Results:** All SNPs in the *ACE* gene were in Hardy-Weinberg equilibrium. Since the *ACE2* gene is located in the X-chromosome, male and female genotypes were analysed separately. Genotypes in the *ACE2* gene were, however, in Hardy-Weinberg equilibrium in the female population. Allele and genotype frequencies did not differ between case and control subjects in any of the polymorphisms. Furthermore, the allele and genotype frequencies were no different between any of the four type 1 diabetic patient groups or between the „extreme" phenotypes (normoalbuminuric vs macroalbuminuric+ESRD). Gender and blood pressure did not affect the results either. All five SNPs of the *ACE* gene contributed to the S-ACE concentration in the same dose-dependent manner. Haplotype analysis are under process for both of the genes.
**Conclusion:** This study does not support an involvement of the *ACE* and *ACE2* genes in diabetic nephropathy in type 1 diabetic patients

## 1080

**Reduction of urinary connective tissue growth factor by losartan in hypertensive Type 1 diabetic patients with diabetic nephropathy**
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**Background and aims**: Connective Tissue Growth Factor (CTGF) is an important prosclerotic cytokine implicated in development of diabetic glomerulosclerosis. Urinary CTGF is reported to be significantly increased in patients with diabetic nephropathy. The present study aimed to investigate the short- and long term effects of angiotensin II receptor blockade by Losartan on urinary CTGF levels in hypertensive type 1 diabetic patients with diabetic nephropathy.
**Materials and Methods:** Seventy-one hypertensive type 1 diabetic patients with diabetic nephropathy were included in the study. After a washout period of four weeks, the patients received Losartan 50 mg, 100 mg and 150 mg o.d. in treatment periods each lasting two months. Thereafter, patients were followed prospectively during treatment with Losartan 100 mg o.d. with a total mean follow-up time of 36 months. At baseline, after two, four and six months and then biannually, urinary CTGF (ELISA; Fibrogen), plasma CTGF, albuminuria (Turbidimetry), glomerular filtration rate (GFR)($^{51}$Cr-EDTA plasma clearance) and 24 hours blood pressure (TM2420 A&D) were determined.
**Results:** Baseline levels of urinary and plasma CTGF were 7076 (5708–8770) ng/24 h (geometric mean (95 % CI)) and 12.7 (7.3) ng/ml (mean (SD)), respectively.
Albuminuria, GFR and arterial blood pressure at baseline were 1152 (937–1416) mg/24 h, 88 (24) ml/min/1.73 m$^2$, 153/80 (9/17) mm Hg respectively.
Losartan significantly reduced urinary CTGF by 21 % (9–31) (95% CI) initially ($p < 0.05$ vs baseline), with no further reduction after increasing dose. The sustained reduction in urinary CTGF was 22 % (12–32) ($p < 0.05$ vs baseline). Rate of decline in GFR during the study was 3.2 (–1.6 – 15.9) ml/min/year (median (range)). Reduction in urinary CTGF was correlated with a lower rate of decline in GFR ($r = 0.23$; $p = 0.05$). Plasma CTGF remained unchanged throughout the study.
**Conclusion:** Our three year study demonstrates that Losartan persistently reduces urinary CTGF excretion, which is associated with a slower rate of decline in GFR.

## 1081

**Is non-albuminuric renal insufficiency in Type 2 diabetes related to an increase in intra-renal vascular disease?**
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**Background and aims:** Traditionally, microvascular disease resulting in a glomerulopathy and an increase in albumin excretion rate (AER) is believed to be the dominant mechanism by which diabetic nephropathy develops. However, recent results have challenged the concept that a decline in renal function in patients with diabetes is always accompanied by an increased AER. The aim of this study was to investigate the role of intra-renal vascular disease in the pathogenesis of non-albuminuric renal insufficiency.
**Materials and methods:** We studied 325 unselected clinic patients who had sufficient clinical and biochemical information to calculate a GFR using the Modified Diet in Renal Disease (MDRD-6) formula, at least 2 estimations of AER and a renal duplex scan to estimate intra-renal vascular resistance, i. e. the parenchymal resistance index (PRI).
**Results:** Overall, 93 (29 %) of patients had renal insufficiency defined as a GFR < 60/ml/min/1.73 m$^2$. Of these patients, 39 (42 %) had normo-, 29 (31 %) had micro- and 25 (27 %) had macro- albuminuria. Macroalbuminuric patients were older and had a lower GFR than normo- and micro- albuminuric patients but there were no significant differences in gender, duration of diabetes, prevalence of macrovascular disease, blood pressures or use of RAS inhibitors between the groups. GFR ($p = 0.0001$) and age ($p = 0.004$) but not AER were the main independent predictors of PRI. The mean PRI values for normo-, micro- and macro- albuminuric patients with a GFR > 60 ml/min/1.73 m$^2$ were all < 0.7. As shown in the table, the renal PRI was elevated to a similar extent for all groups of patients with impaired renal function regardless of their albuminuric status.
**Conclusion:** The pathogenesis of non-albuminuric, as opposed to albuminuric, renal insufficiency in type diabetes is not explained by differences in renal vascular disease as measured by the parenchymal resistance index of the interlobar arteries. The structural basis of non-albuminuric renal insufficiency in type 2 diabetes remains to be elucidated.

Table

| Variable | Normo (n=39) GFR < 60 | Micro (n=29) GFR < 60 | Macro (n=25) GFR < 60 | p |
|---|---|---|---|---|
| Age (years) | 72 ± 1 | 73 ± 2 | 67 ± 2 | 0.02 |
| MDRD-GFR | 45 ± 2 | 46 ± 2 | 35 ± 3 | <0.001 |
| PRI | 0.74 ± 0.01 | 0.73 ± 0.01 | 0.75 ± 0.11 | 0.52 |

## 1082

**Positive correlation between plasma activities of semicarbazide-sensitive amine oxidase and angiotensin-converting enzyme in patients with Type 1 diabetes mellitus.**
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**Background and aims:** Semicarbazide-sensitive amine oxidase (SSAO) ) has been implicated in the pathophysiology of late complications and plasma SSAO has been shown to be elevated in patients with Type 1 diabetes. The mechanism of this rise remains uncertain, but investigations into correlations with other clinical and biochemical parameters might give clues .
**Materials and methods:** We have measured plasma SSAO activities with an established method in a large well-characterized group of 285 patients with Type 1 diabetes. Standard statistical methods were used to investigate correlations with relevant clinical and biochemical parameters.
**Results:** In the total group SSAO was elevated, with 693 ± 196 mU/l (mean ± SD; normal controls 352 ± 102 mU/l). SSAO was higher in the group with late complications ( 727 ± 202 vs 627 ± 164 mU/l, $p<0.001$) and in hypertensive patients ( 782 ± 236 vs 671 ± 180 mU/l, $p<0.001$).
Patients treated with an angiotensin-converting-enzyme (ACE)-inhibitor (n=60) had higher SSAO activities than untreated patients (n=225) (786 ± 218 vs 668 ± 183 mU/l, $p<0.0001$). In univariate analysis a highly significant positive correlation ($p<0.001$, $R=0.27$) was found between plasma SSAO

and ACE activities in patients untreated with ACE inhibitors or angiotensin II receptor antagonists, but SSAO was not different in the I/D polymorphisms of the ACE genotype: II 677 ± 174, ID 681 ± 186 and DD 627 ± 181 mU/l, p=0.172. Other positive correlations of SSAO found were with duration of diabetes (p=0.003, R=0.18), HbA1 c (p=0.007, R=0.16) and renin (p=0.020, R=0.14), while SSAO correlated negatively with angiotensinogen (p=0.002, R=0.19) and body mass index (p=0.020, R=0.14). A multiple regression analysis including these variables in the subgroup untreated with ACE inhibitors resulted in ACE activity (p<0.001) and HbA1 c (p=0.023) as explaining variables.
**Conclusion:** Results suggest that a common factor is involved in the regulation of both plasma SSAO and ACE, apart from the regulation of plasma ACE by the I/D polymorphism of the ACE gene. Both enzymes have been implicated in the pathophysiology of late diabetic complications.

## 1083

**A decline in glomerular filtration rate over time does not reduce insulin sensitivity in patients with Type 1 diabetes**
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**Background and aims:** Abnormalities in glucose metabolism and insulin resistance has since long been recognised in patients with chronic renal failure. More recent studies have shown that insulin resistance may be present even in mild renal impairment. However, less is known about how a decline in glomerular filtration rate (GFR) over time affects insulin sensitivity. In this study we utilised the euglycemic hyperinsulinemic clamp technique to evaluate the impact of a decline in GFR over time in patients with type 1 diabetes.
**Patients and methods:** In a prospective follow-up study 21 patients with type 1 diabetes were re-examined after 5.1 ± 0.4 years (range 4.2–6.2). At baseline 8/21 (38%) patients had no signs of renal impairment, 9/2 (43%) had albuminuria but normal GFR and 4/21 (19%) had a reduced GFR, defined as <75 ml/min/1.73 m$^2$, (56.2 ± 10.8 ml/min/1.73 m$^2$, range 43–65). 17/21 (81%) of these patients displayed a decline in GFR during the follow-up period. GFR was determined by 51Cr-EDTA clearance. Insulin sensitivity was assessed as glucose uptake at steady state during clamp (M-value) and calculated per lean body mass (kg). Ongoing antihypertensive medication was withdrawn two weeks prior to the clamp. Blood pressure (mmHg) was measured without ongoing antihypertensive medication both at baseline and at follow-up.
**Results:** At baseline a significant difference in insulin sensitivity was found between patients with reduced GFR and those with normal GFR (no signs of renal impairment and albuminuria but normal GFR) (m-value; 4.7 ± 2.5 vs. 8.6 ± 3.1 mg/kg LBM/min, p=0.03). At follow-up no significant difference was found between the two groups (m-value; 8.1 ± 2.6 vs. 8.9 ± 1.9, p=0.43). In patients with a decline in GFR during the follow-up period, this reduction (GFR; 15.1 ± 15.9 ml/min/1.73 m$^2$, range 1–69) did not correlated to change in insulin sensitivity (m-value; 0.7 ± 4.3, r=0.36, p=0.15). Interestingly, 4 patients with a reduced GFR at baseline improved their insulin sensitivity significantly (3.43 ± 1.6, p=0.024) during the follow-up period. No significant changes were found in HbA1 c (–0.26 ± 1.2 %, p=0.36) or BMI (–0.28 ± 1.2 kg/m$^2$, p=0.52) in patients with a decline in GFR during the follow-up period. A near-significant reduction in systolic (–10.4 ± 20.9 mmHg, p=0.057) and a significant reduction in diastolic blood pressure (–6.8 ± 10.6 mmHg, p=0.018) were found.
**Conclusions:** In patients with type 1 diabetes a decline in GFR over time does not seem to diminish insulin sensitivity. On the contrary, in patients with reduced GFR at baseline an increase in insulin sensitivity was found. This may be due to effects of intensive treatment with antihypertensives, especially ACE-inhibitors or AT2-receptor blockers, and modern insulin analogues.

# PS 108

# Nephropathy: risk factors, predictors and progression promotors

## 1084

**The relation between nephropathy and dyslipidemia in Type 2 diabetic patients**
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**Background and aims:** Known cardiovascular risk factors may have an important role in diabetic nephropathy. This study was designed to determine the relation between dyslipidemia and albuminuria, in type 2 diabetic patients.
**Materials and methods:** The study involved 600 type 2 diabetic patients who presented to a university diabetic clinic from October 2002 till October 2003. Information was collected in registration form and clinical database. Measurements included FBS, HbA1 c, total, and HDL cholesterol, triglyceride, apolipoprotein B, lipoprotein (a), serum creatinine, and daily urinary albumin excretion (UAE). LDL cholesterol and glomerular filtration rate (GFR) were calculated. UAE was measured by immunoturbidometric method. Patients with ESRD, congestive heart failure, liver disease, positive urinalysis for nitrite and protein and uncontrolled hyperglycemia were excluded. We analyzed the data to find-out the relation among clinical findings, UAE, and GFR and serum lipid profile.
**Results:** A total of 407 patients (108 M & 299 F) completed the study. 255 (63%) of the patients had normal albumin excretion; 131 (32%) had microalbuminurai, and 21 (5%) had macroalbuminuria. The age was 54 ± 11 years (mean ± SD); duration of diabetes 8.8 ± 7.5 years, BMI 27 ± 5.5, SBP 130 ± 19, DBP 80 ± 11 mmHg. Total-cholesterol was 220 ± 65 mg/dl (mean ± SD), triglyceride 219 ± 171, HDL-C 45 ± 15, LDL-C 133 ± 45, apolipoprotein B 111 ± 30, lipoprotein (a) 49 ± 42, FBS 197 ± 73, and Hb A1 c 9.9 ± 2.7 %. Creatinine clearance was 83 ± 28 ml/min. Systolic and diastolic blood pressure had a significant correlation with total and LDL cholesterol and apolipoprotein B. GFR was correlated to total cholesterol (p<0.05), HDL-C (p<0.001), lipoprotein (a) (p<0.001), and duration of diabetes (p<0.01). UAE was correlated with HDL-C (p<0.01) and diabetes duration (p<0.02). HDL-C had also correlation with GFR (p<0.001), and duration of diabetes (p<0.02). The relation between HDL-C and albuminuria was independent of other serum lipoproteins, GFR and blood pressure. There were no correlation between other serum lipoproteins and UAE. The correlation between UAE and duration of diabetes was also independent of other serum lipoproteins, FBS, blood pressure, and GFR.
**Conclusion:** Increased duration of diabetes was a risk factor for diabetic nephropathy as in other studies. Low serum HDL-C may be another risk factor for diabetic nephropathy. Increased duration of diabetes and low HDL-C may predict increased UAE and decreased GFR. Diabetic dyslipidemia by its mutual interaction with hypertension may further predispose to nephropathy.
*Supported by: Endocrine Research Center,Tehran University of Medical Sciences*

## 1085

**Increased levels of cellular adhesion molecules and white blood cell counts are associated with risk of diabetic nephropathy in Type 2 diabetes**
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**Background and aims:** Diabetic nephropathy (DN) and coronary heart disease are considered to reflect manifestation of a generalized vascular disease. Since increased levels of soluble cell adhesion molecules (CAMs) and raised white blood cell counts (WBC) have been shown to predict development of cardiovascular disease in type 2 diabetes (T2DM), we investigated the association between plasma concentrations of sVCAM-1, sICAM-1 and WBC and the risk of DN among T2DM subjects.
**Materials and methods:** In a retrospective cross-sectional study 169 patients (mean age 62 ± 10 yr, diabetes duration 14 ± 10 yr) with T2DM were examined. DN was diagnosed when albumin excretion rates (AER) were > 200 µg/min and/or serum creatinine > 116 µmol/l. CAMs were measured by enzyme-linked immunosorbent assays. Categories of sVCAM-1, sICAM-1 levels and WBC were defined by quintiles. Multivariate logistic

regression analysis was used to evaluate the impact of the different variables on DN risk.
**Results:** In the entire cohort, sVCAM-1, sICAM-1 and WBC were significantly associated with logAER (R = 0.418, P < 0.001; R = 0.275, P < 0.001; R = 0.232, P = 0.004). With the exception of sVCAM and WBC which correlated with age, there were no further associations with conventional risk factors of diabetic nephropathy or atherosclerosis. Compared with the three lowest quintiles, the relative risks for DN among T2DM subjects in the fifth quintiles with sVCAM-1 levels of > 1052 ng/ml, sICAM levels of > 441 ng/ml and WBC of > 8.7 × $10^9$ cells /l were 11.9 (95% CI 4.2 – 33.7), 3.1 (CI 1.4 – 7.2) and 3.6 (CI 1.5 – 8.2), respectively.
**Conclusion:** Levels of sVCAM-1, sICAM-1 and WBC are associated with the risk of diabetic nephropathy in T2DM and thus might be useful for identification of subjects at risk of developing end-stage renal disease.

## 1086

**Association between mannose-binding lectin and vascular complications in Type 1 diabetes**

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**Background and aims:** Complement activation and inflammation have been suggested in the pathogenesis of diabetic vascular lesions. We investigated serum mannose-binding lectin (MBL) levels and polymorphisms in the MBL gene in type 1 diabetic (T1DM) patients with and without diabetic nephropathy and associated macrovascular complications.
**Materials and methods:** Polymorphisms in the MBL gene and serum MBL levels were determined in 199 T1DM patients with overt nephropathy and 192 T1DM patients with persistent normoalbuminuria matched for age, sex, and duration of diabetes, as well as in 100 healthy control subjects.
**Results:** The frequencies of high and low expression MBL genotypes were similar in patients with T1DM and healthy controls. High MBL genotypes were significantly more frequent in diabetic patients with nephropathy than in the normoalbuminuric group, and the risk of having nephropathy given a high MBL genotype assessed by odds ratio was 1.52 (1.02–2.27), $P$ = 0.04. Median serum MBL concentrations were significantly higher in patients with nephropathy than in patients with normoalbuminuria (2306 µg/l [IQR 753–4867 µg/l] vs. 1491 µg/l [IQR 577–2944], $P$ = 0.0003), and even when comparing patients with identical genotypes serum MBL levels were higher in the nephropathy group than in the normoalbuminuric group. Patients with a history of cardiovascular disease had significantly elevated MBL levels independently of nephropathy status (3178 µg/l [IQR 636–5231 µg/l] vs. 1741 µg/l [IQR 656–3149 µg/l], $P$ = 0.02). The differences in MBL levels between patients with and without vascular complications were driven primarily by pronounced differences among carriers of high MBL genotypes ($P$ < 0.0001).
**Conclusion:** Our findings suggest that MBL may be involved in the pathogenesis of micro- and macrovascular complications in type 1 diabetes, and that determination of MBL status might be used to identify patients at increased risk of developing these complications.
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## 1087

**Increased levels of mannan-binding lectin (MBL) in Type 1 diabetic patients with incipient and overt nephropathy**

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**Background and aims:** Diabetic nephropathy is associated with insulin resistance and low-grade inflammation and activation of the complement system may contribute to this cascade. Mannan-binding lectin (MBL) activates the complement system, and elevated MBL concentrations have been observed in normoalbuminuric type 1 diabetic patients. The aim of the study was to assess whether MBL is associated with diabetic nephropathy in type 1 diabetes, and whether there is an association between MBL and low-grade inflammatory markers or insulin resistance.
**Methods:** 193 type 1 diabetic patients from the Finnish Diabetic Nephropathy Study were divided based upon their albumin excretion rate (AER) into three groups. Patients with normoalbuminuria (n=67) had no antihypertensive medication nor signs of cardiovascular disease, while patients with micro- (n=64) or macroalbuminuria (n=62) were all treated with an angiotensin-converting enzyme (ACE) inhibitor. As a measure of insulin sensitivity we used estimated glucose disposal rate (eGDR). MBL was measured by an immunofluorometric assay, CRP by radioimmunoassay and interleukin 6 (IL-6) by high sensitivity enzyme immunoassay.
**Results:** Patients with normoalbuminuria (median [IQR]; 1154 µg/l [180–2202 µg/l]) had lower levels of MBL than patients with micro- (1713 µg/l [719–2784 µg/l]; p=0.034) or macroalbuminuria (1648 µg/l [568–3394 µg/l]; p=0.019). In univariate analyses, there was a significant correlation between MBL and eGDR (r=–0.17, p=0.018), HDL-cholesterol (r=–0.19, p=0.012) and $HbA_{1c}$ (r=0.21, p=0.003), but not between MBL and low-grade inflammatory markers. In multiple regression analysis, $HbA_{1c}$ was the only variable independently associated with MBL (β±SEM; 0.26 ± 0.08; p=0.003).
**Conclusions:** MBL concentrations are increased in type 1 diabetic patients with diabetic nephropathy. MBL was not associated with low-grade inflammatory markers. The predictive value of MBL needs to be assessed.
*Many Thanks to all the physicians and nurses at each FinnDiane center in Finland*

## 1088

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**Progression of nephropathy in Type 2 diabetic patients**

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**Background and aims:** Nephropathy in type 2 diabetes is the single most common cause of end-stage renal disease (ESRD) but the decline in kidney function varies considerably between individuals, and determinants of renal function loss, early in the course of renal disease, have not been clearly identified. We evaluated determinants of the rate of decline in renal function and mortality in type 2 diabetic patients
**Materials and methods:** In a prospective observational study, we followed 227 (60 female) Caucasian type 2 diabetic patients with nephropathy for 6.5 (range:3–17) years from a baseline GFR of 83 (SD:30) ml/min/1.73 $m^2$ with 7(range:3–22) measurements of GFR($^{51}$Cr-EDTA) per patient. We evaluated determinants of 1) rate of decline in GFR, 2) risk of doubling in serum creatinine or ESRD, and 3) mortality using potential risk factors at baseline and during follow-up.
**Results:** The mean (SD) rate of decline in GFR was 5.2 (4.1) ml/min/year. In multivariate regression analysis, higher baseline albuminuria, systolic blood pressure(SBP), hemoglobin A1 c, GFR, age, and degree of diabetic retinopathy were significantly associated with increased rate of decline in GFR($R^2_{adj}$:0.24). During follow-up, elevated mean albuminuria, SBP, hemoglobin A1 c, and lower hemoglobin, heavy smoking and presence of diabetic retinopathy were significantly associated with increased decline in GFR($R^2_{adj}$:0.26). During follow-up, 63 patients had a doubling in serum creatinine or developed ESRD, and 79 patients died, primarily due to cardio-

vascular disease. In Cox regression analysis, higher baseline albuminuria, hemoglobin A1 c and SBP together with lower GFR and hemoglobin were significantly associated with shorter time to doubling of serum creatinine or ESRD. Higher baseline albuminuria, hemoglobin A1 c, SBP and age were significantly associated with increased mortality.
**Conclusion:** Our long-term prospective study of type 2 diabetic patients with nephropathy has revealed several modifiable risk factors of enhanced progression in kidney disease and increased mortality risk.

## 1089

**Plasma NT-proBNP - an independent predictor of mortality in diabetic nephropathy**

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**Background and aims:** Raised N-terminal pro brain natriuretic peptide (NT-proBNP) is independently associated with an increased risk for death in chronic heart failure and acute coronary syndromes in non-diabetic populations. Diabetes and diabetic nephropathy in particular are characterised by an increased risk of cardiovascular morbidity and mortality. This study investigated the prognostic value of NT-proBNP in a large cohort of Type 1 diabetic patients with and without diabetic nephropathy.
**Material and methods:** In a prospective observational follow-up study 198 type 1 diabetic patients with overt diabetic nephropathy (122 men, age (mean(SD)) 41 ± 10 years, duration of diabetes 28 ± 8 years, GFR 75 ± 33 ml/min/1.73 $m^2$) and a matched control group of 188 patients with long-standing type 1 diabetes and persistent normoalbuminuria (114 men, age 43 ± 10 years, duration of diabetes 27 ± 9 years) were followed for 9.3 (0.0–9.5) years. Plasma NT-proBNP concentration was determined by immunoassay at baseline. Endpoints were all cause and cardiovascular mortality.
**Results:** In patients with diabetic nephropathy, plasma NT-proBNP concentration was elevated 110 (5 – 79640) ng/l (median(range)) versus 27 (5 – 455) ng/l in normoalbuminuric patients, $p<0.001$. Circulating NT-proBNP was elevated early in diabetic nephropathy when glomerular filtration rate was still well preserved (>101 ml/min/1.73 $m^2$). During follow-up 51 patients with and 11 patients without nephropathy died, $p<0.001$. Among patients with nephropathy 39 (39%) patients with plasma NT-proBNP above the median (110 ng/l) and 12 (12%) with values below the median died from any cause (unadjusted hazard ratio 3.86 (95%CI 2.02–7.37), $p<0.0001$; covariate adjusted 2.28 (1.04–4.99), $p=0.04$). This lower mortality was attributable to fewer cardiovascular deaths: 31 (31%) and 7 (7%) above and below the median NT-proBNP level respectively (unadjusted hazard ratio 5.25 (2.31–11.92), $p<0.0001$; covariate adjusted 3.81 (1.46–9.94), $p=0.006$).
**Conclusions:** Elevated circulating NT-proBNP is a new independent predictor of the excess overall and cardiovascular mortality in asymptomatic patients with diabetic nephropathy.

## 1090

**Associations of plasma erythropoeitin concentration in patients with Type 2 diabetes**

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**Background and aims:** Diabetes is the most common cause of end-stage renal disease in Kuwait. We postulate that inappropriately low erythropoietin concentration occurs early in the development of nephropathy even in patients without anaemia. This study investigates the relationships between the degree of nephropathy and erythropoietin concentration in patients with type 2 diabetes.
**Materials and methods:** We measured serum erythropoietin (Epo), ferritin and complete blood count in 161 type 2 diabetic patients (93 females and 68 males, mean (SD) age: 58.85 (10.76), diabetes duration: 12.3 (8.14) yrs. Serum creatinine and calculated creatinine clearance (Cockroft-Gault) were used as markers of glomerular filtration rate and urine microalbumin:creatinine ratio was determined to classify patients as normo-, micro- or macro-aluminuric.
**Results:** The mean (95% confidence interval, CI) Epo in patients with normo-, micro- and macroalbuminuria were 7.96 (6.12 9.81) mU/mL; 5.02 (3.74 6.30) and 4.29 (2.35 6.24) mU/mL respectively. Ferritin showed the opposite trend with mean (CI) values of 100.71 (83.54 117.87)ng/mL; 110.58 (80.23 140.92) ng/mL and 161.92 (56.90 266.94) ng/mL in patients with normo-, micro- and macro-albuminuria respectively. Mean Hb was not significantly different between patients with normo- (138 g/L); micro- (136 g/L) and macro-albuminuria (131 g/L) ($p = 0.46$). Spearman correlation analyses showed that erythropoietin was significantly correlated with serum creatinine ($r = -0.16$; $p = 048$); microalbumin:creatinine ratio ($r = -0.18$; $p = 0.04$); Hb ($r = 0.23$; $p = 0.004$). However partial correlation analyses correcting for age and sex showed that Epo correlated with Hb ($p < 0.0001$); MCV ($p < 0001$) and creatinine clearance ($p < 0.001$) only. There was no correlation with age, duration of diabetes and HbA1 c.
**Conclusion:** Epo concentration is inappropriately low in diabetic patients independently of the ferritin and haemoglobin concentrations. There is need to determine serum Epo as low erythropoietin may precede the onset of incipient nephropathy in diabetic subjects.
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# PS 109

# Nephropathy: gene polymorphisms and molecular biology

## 1091

**Paraoxonase 2 polymorphisms are associated with proteinuria in Japanese Type 2 diabetic patients**

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**Background and aims:** Paraoxonase 2 (PON2) gene polymorphisms have been suggested as a risk marker of coronary heart disease in non-diabetic subjects and of vascular complication in diabetic patients. This study was designed to evaluate whether PON2 polymorphisms are associated with diabetic nephropathy in Japanese Type 2 diabetic patients.
**Patients and methods:** PON2 Ala/Gly 148 (PON2-148) and PON2 Cys/Ser 311 (PON2-311) gene polymorphisms were studied in 136 Japanese Type 2 diabetic patients (102 non-proteinurics and 34 proteinurics with retinopathy, with median age of 64 and 64 years old, and median diabetes duration of 13 and 15 years, respectively). Genotyping of the PON2-311 and PON2-148 polymorphisms were made by a PCR method using 3 respective primers based on amplification refractory mutation system (ARMS).
**Results:** The frequencies of genotypes with C (CC+SC) and without C allele (SS) (PON2-311) were 36.3 % and 63.7 % in the non-proteinurics and 81.8 % and 18.2 % in the proteinurics, respectively ($p < 0.05$). Similarly, those with G (GG + GA) and without G allele (AA) (PON2-148) were 36.3 % and 63.7 % in the non-proteinurics and 81.8 % and 18.2 % in the proteinurics, respectively ($p < 0.05$). Presence of PON2-148 C allele was individually associated with the PON2-311 G allele.
**Conclusion:** The results support the hypothesis that PON2 gene polymorphisms are associated with diabetic nephropathy. PON2 gene genotyping can be a clinically-useful genetic marker for predicting the development of nephropathy, only if such an association is proven to be present in a larger number of both Type 1 and Type 2 diabetic patients.

## 1092

**N-acetylation polymorphisms in Type 2 diabetes with and without microvascular complications**

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**Background and aims:** The relationship between genetically determined polymorphic nature of acetylation and susceptibility to diabetes mellitus and its complications have not yet been settled. Some of the studies suggest that fast acetylators, mainly due to the underlying genetic factor, are more susceptible to develop type 1 and type 2 diabetes. Other investigators feel that altered acetylating capacity in diabetes mellitus is a consequence of hyperglycemia due to increased hepatic concentration of acetyl CoA. The aim of the present study was to investigate the acetylator phenotype status in type 2 diabetes with and without microvascular complications in a Bangladeshi population.
**Materials and methods:** The diabetic groups consisted of 35 type 2 subjects without complications (19 women and 16 men, aged 32–73 yrs and duration of diabetes 4–23.50 yrs) and 64 type 2 subjects with complications (42 women and 22 men, aged 30–70 yrs, and duration of diabetes 3–27 yrs). The healthy control group included 21 women and 22 men (aged 20–65 years). Acetylation status was measured by Price-Evans method using sulphadimidine as a probe and percent acetylation was calculated by the Bratton-Marshall procedure.
**Results:** Prevalence of fast acetylators were significantly higher in type 2 diabetes when compared with healthy controls (62.6% vs 41.9%, $\chi 2 = 5.255$ and p=0.022). A slight preponderance of slow acetylators in DM with microvascular complications (42.2%) was observed when compared with DM without microvascular complications (28.6%), but the difference was not statistically significant. $HbA_{1c}$ was significantly higher in DM with microvascular complications and DM without microvascular complications when compared with healthy controls ($HbA_{1c}$, M±SD, 9.17 ± 1.80, 8.76 ± 1.72 & 5.32 ± 0.43, p=0.001 & 0.001 respectively), but there was no significant difference between the two diabetic groups. There was a positive correlation between $HbA_{1c}$ and % acetylation (r=0.304, p=0.001).
**Conclusion:** Fast acetylation status is associated with type 2 diabetes mellitus (but not with microvascular complications) and hyperglycemia seems to be an important determinant for the fast acetylation capacity.

## 1093

**Apolipoprotein B gene contributes to the genetic predisposition to diabetic nephropathy in Type 1 diabetes**

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**Background and aims:** Diabetic nephropathy (DN) is a major long-term complication of type 1 diabetes (T1DM) but many patients tolerate the metabolic abnormalities of diabetes for decades without developing DN, suggesting the presence of genetic predisposition to DN in type 1 diabetes. An association of *APOE* gene polymorphic markers with DN in patients with T1DM and T2DM have been shown in several studies. We hypothesized that the particular genetic characteristics of lipid metabolism can contribute to development of DN and suggested that *APOB* gene can be also involved in the DN development. In this study we investigated an association of 9 bp insertion/deletion polymorphism (*I/D*) in the signal peptide region of *APOB* gene, encoding amino acids –16 to –14, with DN in T1DM patients.
**Materials and methods:** To overcome probable predominance and masking effects of metabolic risk factors on DN phenotype expression, we have formed two groups of T1DM patients: DN+ group (n=65) with the overt diabetic nephropathy and DN- group (n=73) in which all patients had no clinical nephropathy. To identify the alleles of polymorphic marker we used PCR technique and gel electrophoresis.
**Results:** The frequency of *I* allele was higher in DN+ group (0.72) than in DN- group (0.58) whereas the frequency of *D* allele was lower in DN+ group (0.28) than in DN- group (0.42). We have also calculated the odds ratios and found that the carriers of *I* allele and *II* genotype had higher risk (*OR* = 1.91 and 2.11, relatively; $p < 0.034$), whereas the carriers of *D* allele had lower risk of DN (*OR* = 0.52; $p < 0.018$). It is well known that the signal peptides play important role in the effectiveness and correct transportation of protein from cytoplasm to different cell compartments and thus can mediately determine the particular characteristics of lipid metabolism. Perhaps it can explain in what way *I/D* polymorphism of *APOB* gene contributes to the genetic predisposition to DN in T1DM patients. **Conclusions:** Our findings show that *I/D* polymorphic marker of *APOB* gene is significantly associated with DN among Russian T1DM patients and provide evidence implicating *APOB* as a novel susceptibility gene for diabetic nephropathy in T1DM.

## 1094

**Synergistic effect between HSPG and MMP-9 gene polymorphism in the progression and development of Type 2 diabetic nephropathy**

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**Background and aims:** Heparan sulfate proteoglycan (HSPG) is an integral component of glomerular basement membrane, contributing to the selective barrier for albumin filtration. Matrix metalloproteinase-9 (MMP-9) has a ability to degrade type IV collagen, contributing to the metabolism of extracellular matrix (ECM) in glomeruli. Candidate gene study have indicated that both HSPG and MMP-9 gene polymorphism may involve in diabetic nephropathy (DN). To investigate the synergistic effect between HSPG and MMP-9 gene polymorphism on type 2 diabetic nephropathy, a case-control study for 298 type 2 diabetes mellitus (T2DM) with and without nephropathy and 87 non-diabetic control(non-DM) was performed.
**Materials and methods:** The T2DM subjects were classified into three groups: non-DN group, 80 subjects with normal albuminuric; DN-1 group, 129 subjects with microalbuminuric nephropathy and DN-2 group, 89 subjects containing 38 macroalbuminuric nephropathy and 51 renal insufficiency. HSPG gene (G/T) polymorphism were studied by PCR-RFLP method using BamHI digestion and MMP-9 gene (AC)n polymorphism were determined by GeneScan method and the number of (AC)n were sequenced with an ABI Prism 377 Genetic Analyzer.
**Results:** In HSPG gene, T allele carriers, i. e. TT + TG genotypes were significantly elevated in DN-2 group when compared with non-DN or DN-1

group, two tailed Fisher's exact P<0.001 for each, Odds ratio were 4.6(95%CI 1.9–10.9) and 6.2 (95% CI 2.8–13.9) respectively. In MMP-9 gene, AC24 carreiers, i.e. AC24/AC24+ AC24/X (X: other than AC24 alleles) genetypes were obviously increased in DN-2 group, Fisher's two tailed exact P were 0.0096 and 0.023, Odds ratio were 3.6 (95%CI 1.4–9.4) and 2.4 (95%CI 1.1–5.0) respectively.
Combined genotype analysis showed that the TT or TG paired with AC24/AC24 or AC24/X was significantly increased in DN-2 group(19.1%) than non-DN (6.2%) and DN-1(3.1%) group, Fisher's two tailed exact P were 0.020 and 0.0001, Odds ratio were 3.5 (95%CI 1.2–10.1) and 7.4(2.4–22.8) respectively. After adjustment for multiple comparisons, the positive association of the specific combined genetype, TT or TG + AC24/AC24 or AC24/X with DN-2 remains significant when compared with DN-1(P=0.0009), but not with non-DN(P>0.05).
**Conclusion:** These results suggest: 1. HSPG T-allele and MMP-9 AC24 allele were risk factors of severe type 2 diabetic nephropathy respectively. 2. Patients with HSPG TT or TG genotype exhibits a synergistic effect with MMP-9 AC24/AC24 or AC24/X genotype on the progression and development of severe type 2 diabetic nephropathy in Chinese.
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## 1095

**Impact of genetic and non-genetic factors on development and progression of diabetic nephropathy to end stage renal disease (ESRD) in Type 1 diabetes**

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**Background and aims:** Diabetic nephropathy (DN) is known to have multiple risk factors: chronic hyperglycemia, renal and systemic hypertension as well as genetic factors. The aim of our study was to evaluate the impact of genetic and non-genetic factors on development and progression of DN to ESRD in type 1 diabetes mellitus (DM). To study genetic susceptibility we have chosen polymorphic markers of genes involved in production of angiotensin II and nitric oxide – the main factors regulating renal and systemic haemodynamics.
**Materials and methods:** To overcome the masking effects of metabolic factors (e. g. hyperglycaemia and DM duration) we used non-overlapping criteria for group formation: 1) Group 1 ("DN-"), n=66 - patients with normal urinary albumin excretion rate (UAER< 30 mg/24 h) and DM duration > 20 yr. 2) Group 2 ("DN+"), n=63 – patients with overt DN (UAER > 300 mg/24 h) and DM duration < 15 yr. 3) Group 3, n=96 – healthy subjects from general population. All "DN+" patients were divided into two subgroups by serum creatinine (Scr) level: 1) "ESRD+", n=31 – Scr > 110 μ mol/L (236,0 ± 146,7); 2) "ESRD-", n=32 – Scr< 110 μ mol/L (86,8 ± 13,7). Glomerular filtration rate (GFR) was calculated by Cockcroft-Gault method. Polymorphic markers: *I/D* in angiotensin I-converting enzyme gene (*ACE*); *A(-1903G)* in chymase gene (*CMA1*); *M235T* and *T174M* in angiotensinogen gene (*AGT*); *A1166C* in gene encoding angiotensin II receptor, subtype1 (*AT2R1*); *Glu298Asp* and *ecNOS4a/4b* in endothelial NO-synthese gene (*NOS3*) were studied using PCR. Significance of differences in allele/genotype frequencies was assessed by Fisher's exact test.
**Results:** We observed increased frequency of genotype *II* and decrease of genotype *DD* in ACE gene in group "DN-" compared to "DN+" and healthy subjects (36,4vs.23,8vs.13,5%,*OR*=0,55; 18,2vs.20,6vs.41,7%,*OR*=1,17, respectively), and highly significant differences in *ecNOS4a/4b* genotypes in "DN+" and "DN-": *4a/4b* (76,2vs.47,0%,p<0,01,*OR*=3,61) and *4b/4b* (22,2vs.51,5%,p<0,001,*OR*=0,27). There were no significant differences of alleles/genotypes of all polymorphic markers between "ESRD+" and "ESRD-" as well as in sex distribution (m/f: 17/12vs.13/19), age (26,7 ± 6,2vs.25,2 ± 6,8), age at DM onset (13,4 ± 6,8vs.13,0 ± 6,2), DM duration (13,4 ± 2,4vs.12,1 ± 2,9), HbA1 c (9,5 ± 1,9vs.10,1 ± 2,0) and serum levels of cholesterol (6,6 ± 2,3vs.5,9 ± 1,7) and triglycerides (2,5 ± 1,5vs.2,1 ± 1,3). The GFR decline was 11,2 ml/min/1,73 m$^2$/yr in "ESRD+" vs.1,3 ml/min/1,73 m$^2$/yr in "ESRD-" which was strongly associated with BP level and use of antihypertensive therapy . The "ESRD+" patients had an average BP 145/93 mmHg vs. 124/79 mmHg in "ESRD-" (p< 0,01); retrospective analysis showed that 84,4% of "ESRD-" used ACE inhibitors (ACEI) , 9,4% - other antihypertensive treatment and 6,2% had not any antihypertensive therapy, while in "ESRD+" group only 55,2% used ACEI (p< 0,05), 13,8% – other therapy and 31,0% – had no antihypertensive treatment (p< 0,05).
**Conclusion:** In type 1 diabetes genetic susceptibility to DN onset might be attributed to *ID/ACE* and *ecNOS4a/4b* in *NOS3* gene, while progression of DN to ESRD is determined mainly by haemodynamic factors such as systemic hypertension. Lack of ACEI use in DN is another significant risk factor for rapid progression to ESRD.

## 1096

**A novel variant in exon 8 of natriuretic peptide clearance receptor (NPRC) gene is associated with progression of nephropathy in Type 1 diabetic patients**

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**Background and aims:** The clearance receptor for natriuretic peptides (NPRC) plays a key role in the regulation of plasma levels and biological effects of natriuretic peptides, which have been implicated in the onset and progression of diabetic nephropathy. The NPRC gene structure has recently been published. We searched an association between genetic variations at NPRC gene locus and diabetic nephropathy.
**Material and methods:** We carried out a systematic search for polymorphisms in the NPRC gene coding sequence (8 exons) by direct sequencing of the DNA of 48 healthy subjects. Then we genotyped by Restriction Fragment Length Polymorphism (Tsp45I) for the polymorphism we detected 301 type 1 diabetic patients of the SURGENE study, a prospective observational follow-up study carried out in Angers, France, from 1989 to 1999. Patients were classified for nephropathy as follows (number at inclusion): absent (244), incipiens (microalbuminuria, 33), established (macroalbuminuria,18) and advanced (plasma creatinine>150μM, 6). The outcome variable was the occurrence of a renal event, defined as the progression to a further stage of nephropathy. Time-to-first-renal event curves were generated by Kaplan-Meyer estimation according to genotypes for the exon 8 polymorphism (AA vs AG or GG; AG and GG were grouped because of the low G allelic frequency) and compared using the Log-rank test. Cox's proportional hazards model was used to adjust for other prognostic variables (HbA1 c, Systolic Blood Pressure, duration of diabetes, gender and stage of nephropathy at inclusion).
**Results:** The median duration of follow-up was 6 years (2–10). 53 renal events occurred. The sequencing allowed detect a single nucleotide polymorphism in exon 8 (A80G, numbering from first nucleotide in exon 8). The allelic frequencies in SURGENE subjects were A: 80%, G: 20%. The distribution of genotypes was in Hardy-Weinberg equilibrium. The A80G polymorphism was not associated with the initial stage of nephropathy. It was associated with nephropathy progression: log-rank (AA vs AG or GG), 5.22, p=0.022, the G allele being associated with a worse prognosis. After adjustment for other prognostic factors, the risk of progression of nephropathy remained significantly associated with the A80G polymorphism: adjusted hazard ratio 2.1 (95% confidence interval: 1.1–3.9), p=0.028. No interaction with glycaemic control was found.
**Conclusion:** We conducted a systematic analysis of the NPRC gene and identified a novel variant in exon 8. This single nucleotide polymorphism (a substitution of a A for a G) was associated with the progression of diabetic nephropathy in a prospective follow-up study of type 1 diabetic patients. To our knowledge, this is the first study involving the NPRC gene in diabetic nephropathy.
*Supported by ALFEDIAM and Centre Evian Pour l'Eau*

# PS 110

# Nephropathy: microalbuminuria, pathophysiology

## 1097

**Glomerular hyperfiltration results from primary changes in proximal tubular sodium handling but not in volume expansion in Type 1 diabetes mellitus**

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**Background and aims:** Glomerular hyperfiltration is likely to be a requisite for progressive diabetic nephropathy. Although specific mechanisms have not been fully delineated, it has been proposed that hyperfiltration is the consequence of systemic volume expansion in diabetes. However, in recent years it was hypothesized that a primary defect in proximal tubular sodium reabsorption may lead to glomerular hyperfiltration via the tubuloglomerular feedback mechanism (tubular-hypothesis). Our objective was to study glomerular (hyper)filtration in type 1 diabetes in relation to systemic volume expansion and tubular sodium handling.
**Materials and methods:** We have studied 54 normoalbuminuric patients with type 1 diabetes (DP) and 50 healthy controls (C). Glomerular filtration rate (GFR) was measured by inulin clearance. Proximal and distal sodium reabsorption were calculated according to standard formulas. Plasma volume, measured by $^{125}$I-albumin method, atrial natriuretic peptide (ANP) and the second messenger c-GMP were used as markers of extracellular volume expansion.
**Results:** Glomerular filtration rate was higher in DP (GFR 121 ± 2 vs 107 ± 2 mL/min/1.73 m$^2$, $P$<0.001). There were no differences in plasma volume between both groups (2966 ± 81 in DP vs 2953 ± 81 ml in C, NS). Surprisingly, plasma atrial natriuretic peptide was lower in DP (7.6 ± 0.4 vs 8.8 ± 0.5 nmol/L, $P$=0.04) but no significant differences were found in c-GMP levels. The fractional proximal reabsorption of sodium was significantly increased in DP (fPRNa$^+$(%) 90.3 ± 0.3 vs 89.0 ± 0.3 in C, $P$<0.01). There were no differences in distal sodium reabsorption or distal sodium load (≈ macula densa concentration of NaCl) in both groups.
**Conclusion:** Our data suggest that the primary event in diabetic glomerular hyperfiltration is an increase in proximal tubular sodium reabsorption. They do not give support to the hypothesis that systemic volume expansion or ANP mediate glomerular hyperfiltration in normoalbuminuric type 1 diabetes patients.
*Supported by: Dutch Diabetes Research Foundation*

## 1098

**Is microalbuminuria a reliable indicator of underlying renal reserve in diabetes?**

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**Background and aim:** Diabetic nephropathy is characterised by progressive increase in albuminuria and fall in glomerular filtration rate. We evaluated relationship between calculated glomerular filtration rate (cGFR) and albuminuria measured as Albumin/Creatinine ratio (ACR) in our diabetes population.
**Methods:** Data from the Wolverhampton district diabetes register was utilised. Of the 4548 individuals with diabetes who attended over an 18-month period, 4412 had cGFR data available and were studied. cGFR was calculated using Cockroft and Gault equation. Microalbuminuria was measured in spot morning urines as ACR with a threshold of 3.5 mg/mmol taken as abnormal. Statistics were performed using SPSS version 11.0.
**Results:** Seventy eight percent of the population had type 2 diabetes and 87% had normal serum creatinine. The cGFR was >90, 60–90, 30–60 and <30 ml/min in 1006 (23%), 1966 (45%), 1341 (30%) and 99 (2%) individuals respectively. Overall, with progressive fall in cGFR (>90, 60–90, 30–60 and <30 ml/min), mean age [43 ± 10, 60 ± 9, 72 ± 8, 71 ± 14 years], duration [10 ± 8, 11 ± 9, 13 ± 9, 16 ± 10 years], systolic BP [138 ± 20, 146 ± 22, 151 ± 23, 152 ± 26 mmHg], ACR [10 ± 41, 9 ± 31, 20 ± 61, 116 ± 180 mg/mmol], serum creatinine [83 ± 10, 92 ± 13, 114 ± 25, 282 ± 173 µmol/l] and proportion of those with hypertension [57, 77, 87, 88%] and microalbuminuria [29, 30, 58, 78%] all rose significantly (p<0.001). The direct correlation between cGFR and inverse serum creatinine was strong and significant (r=0.65, p<0.001) while that between cGFR and ACR was weak and clinically meaningless. A significant number of individuals even in the lowest cGFR group had normoalbuminuria.
**Conclusion:** Microalbuminuria status on its own does not appear to give a clinically meaningful reflection of underlying renal reserve in individuals with diabetes.
*Supported by: South Staffordshire Medical Foundation, UK*

## 1099

**Serum and urinary adiponectin concentrations were increased in diabetic nephropathy**

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**Background and aims:** Adiponectin(Ad) is presumed to be involved in the pathogenesis of atherosclerosis and insulin resistance. Serum Ad concentrations reported to be elevated in end stage renal failure patients. But the exact role of Ad in the pathogenesis of Diabetic Nephropathy(DN) is not known.
In this study we purposed to elucidate the relationship between serum and urinary Ad concentrations and stages of DN and reveal the relationship between serum and urinary Ad concentrations and several known risk factors of DN.
**Materials and methods:** Total 114 type 2 diabetic patients who did not have non-diabetic kidney diseases were enrolled and divided into three groups: a normoalbuminuric diabetic group (ACR (urinary albumin/creatinine ratio) <30 mg/g, n=67), a microalbuminuric diabetic group (ACR 30 – 299 mg /g, n=34) and an overt proteinuric diabetic group (ACR≥300 mg/g, n=13). Plasma and urinary concentrations of Ad were measured in these subjects by radioimmunoassay (Linco, USA). Urinary Ad levels were expressed relative to the urinary creatinine content as Ad(µg/g Cr).
**Results:** Serum and urinary Ad concentrations increased as the DN stages advanced. Serum Ad concentrations were higher in the proteinuric and microalbuminuric groups than normoalbuminuric group (log Ad, 2.88 ± 0.67, 2.63 ± 0.51 vs. 2.25 ± 0.68, p=0.024, p=0.027, repectably). Urinary Ad levels were also higher in proteinuric group than microalbuminuric and normoalbuminuric groups. (log Ad, 2.09 ± 1.00 vs. 1.39 ± 0.80, 1.12 ± 0.44, p=0.003, p< 0.001, repectably).
Serum Ad concentrations were correlated with urinary ACR (r=0.24, p=0.017), body weight(r=–0.37, p =0.009), age (r=0.35, p<0.001), DM duration (r=0.27, p=0.019), serum albumin(r=–0.36, p=0.002), serum cholesterol (r=0.28, p=0.004), ALT (r=–0.32, p<0.001), BUN(0.24, p=0.015), and CCR(r=–0.34, p=0.018). Urinary Ad also showed positive relationship with ACR (r=0.48, p<0.001), age (r=0.25,p=0.001), diabetes duration(r=0.26, p=0.048), waist circumference (r=–0.58, p=0.012), and serum albumin (r=–0.26, p=0.043). ACR was found to be correlated with urinary Ad (r=0.48,p<0.001), serum Ad (r=0.24, p=0.017), DM duration (r=0.25, p=0.021), albumin (r=–0.24, p=0.018), and Bun (r=0.22, p=0.021).
By linear regression analysis, urinary ACR as a dependent variable, only urinary Ad were included in the model (adjusted $R^2$=25.5 %).
**Conclusion:** In the present study, serum and urinary Ad concentrations increased according as the DN stages were advanced. Urinary Ad had a positive relationship with ACR more than serum Ad. Urinary Ad was an independent variable that determines urinary ACR. Based on the current concept that Ad possesses anti-inflammatory and anti-atherosclerotic effects, this increased concentration in both serum and urine in diabetic nephropathy might reflect some kind of compensatory mechanism to attenuate atherogenic and thrombogenic component of nephropathy. Follow-up study is strongly needed to elucidate whether Ad has protective role or accelerating role on diabetic nephropathy progression.

## 1100

**The association between circulating monocyte chemoattractant protein-1 and urinary albumin excretion in patients with Type 2 diabetes**

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**Background and aims:** The current study was designed to investigate, in patients with type 2 diabetes, whether circulating MCP-1 serves as a candi-

date marker for atherosclerosis by comparison with various established markers: serum high-sensitivity C reactive protein (hsCRP), plasma fibrinogen, and combined carotid artery intimal-medial thickness (IMT). In addition, an association was investigated between circulating MCP-1 and urinary albumin excretion (UAE), reflecting the extent of diabetic renal microangiopathy.
**Materials and methods:** We studied 70 inpatients with poor glycemic control. Serum MCP-1 was measured by enzyme-linked immunosorbent assay. Patients were classified by UAE into three groups: normoalbuminuria, below 30 mg/g of creatinine (Cr), microalbuminuria, 30 to 300 mg/g. Cr, or macroalbuminuria, over 300 mg/g. Cr.
**Results:** Serum MCP-1 for all subjects, men, and women were 280.0 ± 78.9 pg/mL, 269.0 ± 68.8 pg/mL and 294.9 ± 87.9 pg/mL, respectively, showing no difference between genders. There were no correlations between MCP-1 and hsCRP, fibrinogen, or carotid artery IMT. No correlation of MCP-1 was observed with age, duration of diabetes, fasting plasma glucose, hemoglobin $A_{1C}$, body mass index, diastolic blood pressure or serum lipid concentrations, but significant correlations were detected with systolic blood pressure ($R = 0.2723$, $P = 0.0225$) and with $\log_{10}$-transformed UAE ($R = 0.3343$, $P = 0.0047$). Patients with macroalbuminuria showed significant higher circulating MCP-1 than those with normo- or microalbuminuria ($P = 0.0060$, $P = 0.0264$, respectively). By stepwise regression analysis, only UAE independently predicted serum MCP-1 ($\beta = 0.3700$, $P = 0.0020$).
**Conclusion:** The present data suggest that MCP-1 is not a marker for atherosclerosis in patients with type 2 diabetes, and could be influenced significantly by the progression of diabetic nephropathy.

## 1101

**Reactive oxygen species production by polymorphonuclear neutrophils is increased after meal in Type 1 diabetic patients without microalbuminuria**

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**Background and aims:** Postprandial hyperglycemia and oxidative stress play an important role in the development of late diabetic complications. Activated polymorhpnuclear neutrophils (PMN) are an important source of reactive oxygen species (ROS) The aim of this study was to evaluate the influence of low fat meal (30 g carbohydrates, 6 g fat, 8 g proteins) on ROS production by PMN in type 1 diabetic patients with or without microalbuminuria.
**Materials and methods:** The study was performed in 15 type 1 diabetic patients with microalbuminuria (MA+) (8 women and 7 men, aged 27.5+8.7 years, duration of diabetes 10.9+4.9 years, HbA1 c 7.6+1.2 %, FPG 10.4+3.3 mmol/l, 2 hPPG 11.6+3.4 mmol/l, total cholesterol 5.2+0.7 mmol/l) and 15 type 1 diabetic patients without microalbuminuria (MA-) (8 women and 7 men, aged 27.6+9.1 years, duration of diabetes 6.5+4.9 years, HbA1 c 8.0+1.4%, FPG 9.8+2.3 mmol/l, 2 hPPG 10.6+3.4 mmol/l, total cholesterol 5.2+0.8 mmol/l). PMN were isolated from the blood by single-step gradient centrifugation and resuspended in Hanks Balanced Salt Solution to 5 × 106 cells/ml. Superoxide anion ($O^{2-}$) and hydrogen peroxide ($H_2O_2$) production by PMN acc. to Babior et al. and Pick et al.
**Results:** We observed significant higher $O^{2-}$ and $H_2O_2$ production by PMN in subjects MA+ than MA-. We noticed higher ROS production by PMN after meal, but only statistically significant $O^{2-}$ production in diabetic patients MA-.

Mean+SD, *p<0.05 (Mann-Whitney test), **p<0.05 (Wilcoxon test)

| PMN's function | MA+ Fasting | MA+ Postprandial | MA- Fasting | MA- Postprandial |
|---|---|---|---|---|
| $O^{2-}$ (nmol/5x10$^6$ PMN/30 min.) | 6.43+2.33* | 7.21+3.03 | 4.53+2.08 | 6.81+2.33** |
| $H_2O_2$ (nmol/5x10$^6$ PMN/30 min.) | 23.57+11.7* | 25.59+11.69 | 14.88+6.30 | 15.68+6.17 |

* MA+ vs MA-, ** postprandial vs fasting

**Conclusion:** The obtained results show enhanced ROS production by PMNs in type 1 diabetes with nephropathy. The meal seems to be increased ROS production by PMNs especially in type 1 diabetic patient without diabetic complications.

# PS 111

# Nephropathy: experimental pathophysiology

## 1102

**Macrophage scavenger receptor-A promotes infiltration of macrophage into kidney in experimental diabetic mice**

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**Background and aims:** Macrophage scavenger receptor-A (SR-A) is the multiligand and multifunctional receptor which is expressed on macrophage. SR-A recognizes advanced glycation endproducts (AGEs) as well as modified LDL. Moreover, SR-A plays a role in migration of macrophages. Infiltration of macrophages is one of the characteristic features of diabetic nephropathy. We have recently revealed that SR-A knockout (KO) mice are protective against renal injuries after induction of diabetes. In this study, we aimed to clarify the role of SR-A in the pathogenesis of diabetic nephropathy using SR-A KO mice and cultured cells.
**Materials and methods:** We induced diabetes in 8-week-old male SR-A KO mice and wild type (C57BL/6J) mice by injection with streptozotocin. Mice were divided into 4 groups: 1) Diabetic wild type mice (DM-WT) 2) diabetic SR-A KO mice (DM-KO), 3) non-diabetic wild type mice (ND-WT), 4) non-diabetic SR-A KO mice (ND-KO). Mice were killed at 6 months after induction of diabetes, and kidneys were harvested under anesthesia. Blood pressure, HbA1 c, total cholesterol, creatinine clearance (Ccr), urinary albumin excretion (UAE) were evaluated. Glomerular size, mesangial matrix area and interstitial fibrosis were evaluated by morphometry. The number of macrophages was examined by immunohistochemistry. Cell adhesion assays were performed using THP-1 cell (monocyte/macrophage), HUVEC (endothelial cell) and the culture dishes coated with type IV collagen. We evaluated the inhibitory effect of anti-human SR-A monoclonal antibody (mAb) on the attachment of THP-1 cells on type-IV collagen coated dish. In addition, we examined the inhibitory effect of anti-SR-A mAb on the adhesion between THP-1 cells and HUVEC.
**Results:** There was no significant difference in HbA1 c, blood pressure and Ccr between DM-WT and DM-KO. DM-WT presented increased UAE, glomerular hypertrophy and mesangial matrix expansion ($p<0.05$ vs. ND-WT ). These findings are ameliorated in DM-KO ($p<0.05$ vs.DM-WT). In DM-KO, the number of infiltrated macrophages in glomeruli and interstitium was remarkably reduced ($p<0.05$ vs. DM-WT). Additionally, interstitial fibrosis was also supressed in DM-KO. Cell adhesion assay revealed that anti-SR-A mAb significantly decreased the attachment of THP-1 cells on type IV collagen (inhibition rate was 55% as compared with irrelevant antibody). However, anti-SR-A mAb didn't interrupt adhesion between THP-1 cells and HUVEC.
**Conclusion:** Renal injuries were ameliorated in diabetic SR-A deficient mice as compared with diabetic wild type mice. Infiltration of macrophage into renal glomeruli and interstinum was remarkably reduced in SR-A deficient mice as compared with wild type mice after induction of diabetes. Cell adhesion assays revealed that SR-A mediates the attachment of macrophages on type IV collagen, which is a major component of basement membrane and masangial matrix. These results suggest that SR-A dependent migration of macrophages plays important roles in the pathogenesis of diabetic nephropathy.

## 1103

**Induction of pro-inflammatory genes in the kidney in early phase of diabetic nephropathy**

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**Background and aims:** Infiltration of macrophage is prominent in the kidneys of diabetic patients and animals. We previously revealed that intercellular adhesion molecule-1 (ICAM-1) is up-regulated and mediates macrophage infiltration into diabetic kidney. Furthermore, we have shown that ICAM-1 deficient mice were protected from renal injuries after induction of diabetes. Infiltration of macrophages, renal and glomerular hypertrophy, mesangial matrix expansion and albuminuria were suppressed in

ICAM-1 deficient mice as compared with wild type mice at 6 months after induction of diabetes. These data suggest that inflammatory processes are involved in the pathogenesis of diabetic nephropathy. In this study, we aimed to clarify the role of inflammatory processes in the development of diabetic nephropathy using ICAM-1 deficient mice. We induced diabetes in ICAM-1 deficient mice and wild type mice, and evaluated the changes of gene expression profile in the kidney using DNA microarray technique.
**Materials and methods:** Eight-week-old male ICAM-1 deficient mice and wild type (C57BL/6J) mice were used. Diabetes was induced by injection with streptozotocin. Mice were divided into 4 groups: 1) Diabetic ICAM-1 deficient mice (DM-KO), 2) diabetic wild type mice (DM-WT), 3) non-diabetic ICAM-1 deficient mice (ND-KO) and 4) non-diabetic wild type mice (ND-WT). Mice were killed at 2 weeks and 3 months after induction of diabetes and the kidneys were harvested. Blood glucose, HbA1 c, systolic blood pressure, serum creatinine and urinary albumin excretion (UAE) were measured. DNA microarray system (CodeLink DNA array system, Amersham Biosciences, USA) was used to evaluate the expression of 10,012 genes in the kidneys.
**Results:** There was no significant difference in HbA1 c, blood pressure and UAE between DM-WT and DM-KO at 2 weeks after induction of diabetes. Macrophages were increased in renal tissues of DM-WT and suppressed in DM-KO. In the results of DNA microarray study at 2 weeks, we selected the genes that are increased in DM-WT than in ND-WT and decreased in DM-KO than in DM-WT. This gene group included the surface molecules of macrophage (CD68 and MHC class II), chemokines (RANTES, Platelet factor 4, MIG, IP-10, MCP-5 and MIP-1γ) and cytokines and signaling molecules including TGF-β1 and STAT-1. UAE was increased in DM-WT and suppressed in DM-KO as compared with DM-WT at 3 months. Similar changes of proinflammatory genes were observed in the kidneys at 3 months. Extracellular matrix genes such as type VI collagen and lumican were also increased in DM-WT and decreased in DM-KO as compared with DM-WT.
**Conclusion:** Many kinds of proinflammatory genes were induced in the mouse kidneys in early phase after induction of diabetes. These proinflammatory genes were suppressed in ICAM-1 deficient mice resulting in amelioration of renal injuries. These findings suggest that inflammatory process is occurred in early phase of diabetic nephropathy and accelerate renal injuries through enhancement of TGF-β pathway.

## 1104

**Decreased matrilysin (MMP-7) expression and matrix accumulation in human diabetic nephropathy: role of AGE formation**
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**Background and aims:** Diabetic nephropathy is characterised by an increase in the accumulation of extracellular matrix (ECM). High glucose concentration is known to have direct and indirect effects, via glycation on gene expression of mesangial cells. Whilst the direct effect of high glucose is well known the pathophysiology of the indirect actions is incompletely described. For this reason we used micro array analysis to identify new genes that may play a role in this response.
**Methods:** To investigate the effect of glycated albumin on gene expression by mesangial cells (MC), RNA was isolated from MC cultured for 48 hours in media containing either 0.1% albumin (Cont), 0.1% glycated albumin (Galb) or 0.1% albumin in which glycation had been inhibited by co-incubation with aminoguanidine (Galb+AG) and examined using a DNA microarray with 8600 genes. Muliple comparisons were performed and changes in expression were accepted at the 4 fold level. To confirm results obtained in the *in vitro* studies RNA was isolated from glomeruli obtained from kidneys from the following groups of rats control (C:n=8), diabetic (D:n=8) and D+AG (n=8) and from human kidney biopsy specimens (non diabetic: n=8 and diabetic: n=7) and the gene expression examined by real time RT-PCR. Immunohistochemistry was used to study protein concentration in kidney biopsy samples from control (n=6) and diabetic (n=8) baboons obtained after 8 years of diabetes. Staining intensity was scored on a scale of 1–3 (1- least to 3 most intense) by 2 observers blinded to the source to the tissue.
**Results and Conclusions:** Micro array and subsequent real time RT-PCR analysis demonstrated that Galb caused a decrease (>4 fold) in the expression of matrilysin (MMP-7), a metalloproteinase known to degrade fibronectin and laminin. This decrease was ameliorated by prevention of glycation of albumin with AG. In vivo in rodents, diabetes induced a 60% decrease in MMP-7 mRNA which was preventable by AG. Similarly, the expression of MMP-7 was decreased in human diabetic kidney samples by 40% when compared with non-diabetic controls. Furthermore immunohistochemical analysis showed that MMP-7 expression was decreased whilst fibronectin was increased in glomeruli of diabetic baboons when compared with age matched control animals.
Using the microarray technique we have identified MMP-7 as a novel gene affected by exposure to glycated proteins. This decreased expression of MMP-7 was also observed in diabetic kidney tissue from human and rodent sources. In addition decreased MMP-7 correlated with an increase in fibronectin expression. Together these results suggest that MMP-7 plays a role in ECM accumulation in diabetic nephropathy.
*Supported by: JDRFI*

## 1105

**Age-dependent glomerular lesions in galectin-3/AGE-receptor-3 knockout mice**
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**Background and aims:** We previously reported that mice lacking galectin-3, an (anti)adhesive and growth-regulating lectin with advanced glycation endproduct (AGE)-binding properties, are more prone to develop diabetic glomerular disease than those expressing this lectin (Pugliese G. et al., *FASEB J*, 15:2471, 2001). Accelerated glomerulopathy was associated with more marked increases in circulating and renal tissue AGE levels, thus suggesting that it was attributable to the lack of galectin-3 AGE-receptor function . To further verify this hypothesis, we evaluated the development of glomerular lesions in aging galectin-3 knockout (KO) vs. wild type (WT) mice and their relation to the increased AGE levels characterizing the aging process.
**Materials and methods:** KO and WT mice of both sexes were sacrificed at 2, 6, 12, 18 and 24 months of age for the assessment of kidney function (serum creatinine – by the Jaffe method – and proteinuria – by the Bradford method) and structure (morphometric evaluation of glomerular and mesangial area and semiquantitative assessment of glomerular sclerosis index, GSI), together with serum and kidney AGE levels (by ELISA), renal content of the oxidation product 4-hydroxy-2-nonenal (HNE, by immunohistochemistry) and kidney cortex gene expression of the extracellular matrix components fibronectin, laminin B1 and collagen IV a1 chain and the prosclerotic cytokine TGF-b1 (by competitive RT-PCR).
**Results:** Galectin-3 KO mice showed increased proteinuria, GSI, glomerular and mesangial fractional area, as compared with age-matched WT mice, with increaments that were significant only at 18 and, particularly ($p<0.001$), 24 months (proteinuria: $4.63 \pm 1.24$ vs. $2.99 \pm 0.63$ μg/mg creatinine; GSI: $1.83 \pm 0.21$ vs. $1.40 \pm 0.18$; mean glomerular area: $4333 \pm 320$ vs. $3589 \pm 168$ $\mu m^2$; mesangial fractional area: $38.10 \pm 2.88$ vs. $31.60 \pm 2.62$). Starting at 12 months, there was also a significant increase in kidney cortex matrix and TGF-β1 gene expression (by 25–53% at 2 years, $p<0.001$) and, at 24 months of age, a detectable increment of glomerular HNE content ($19.73 \pm 3.02$ vs. $8.751 \pm 2.57$ % of glomerular area, $p<0.001$). Circulating and renal AGE levels were higher in KO vs. WT mice ($3.78 \pm 0.81$ vs. $2.59 \pm 0.42$ U/ml serum and $7.87 \pm 1.33$ vs. $5.45 \pm 0.56$ U/mg tissue), though they were much lower than those previously found in diabetic mice, as lower were the differences between the two genotypes.
**Conclusion:** These data suggest that a moderate but persistent accumulation of AGEs, as that characterizing the aging process, is associated with the development of significant renal functional and structural changes in galectin-3 KO mice. These changes, however, are less marked and occur more slowly than those observed in the same mouse model when rendered experimentally diabetic, a condition characterized by extensive AGE formation and accumulation.
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## 1106

**Renal activity and expression of akt kinase in different models of diabetes (DM)**
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**Background and aims:** Akt is a part of signaling cascades transducing signals triggered by growth and vasoactive factors, including insulin. Akt is involved in regulation of carbohydrate metabolism, cell growth and viability, and in the control of local hemodynamics. Impaired insulin signaling

via Akt in the skeletal muscle, liver and adipose tissue has been implicated in the pathophysiology of insulin-resistant states including Type 2 diabetes (DM2). In contrast to DM2, Type 1 DM (DM1) is not associated with a primary defect in Akt signaling. However, in DM 1 Akt activity could be modulated by opposing actions of hyperglycemia and insulin treatment. Kidneys are one of targets of insulin actions. Akt-dependent insulin effects could contribute to changes in renal morphology, hemodynamics and tubular function in DM. To address this issue, renal cortical Akt activity and expression was determined in obese Zucker rats (ZDF), a model of DM2, in streptozotocin diabetic rats (STZ), a model of DM1, and in appropriate controls, in relation to plasma insulin levels.

**Materials and methods:** ZDF and age-matched controls without metabolic defect (Zucker lean, ZL) were studied at 4 (ZDF4) and 12 weeks (ZDF12) of age. STZ rats (Wistar) were randomized into 3 groups treated with no insulin, and with 4IU and 12IU of insulin/day (STZ0, STZ4, STZ12) to achieve different levels of metabolic control and insulinemia. The measurements were performed after 4 weeks of DM, and the results compared to age-matched non-diabetic controls (C). To determine Akt activity, tissue homogenates were immunoprecipitated with anti-Akt antibody, and incubated with ATP and GSK-3 as a substrate. The GSK-3 phosphorylation was determined by western blotting (WB) as a measure of Akt activity. In addition, protein expressions of active phosphorylated Akt (Serine 473, P-Akt) and total Akt (WB) were also measured. Densitometric analyses of resulting blots, in relation to expression of ubiquitous protein, are presented as fold control (ZL or C=1). Plasma insulin concentrations (P-Ins) were analyzed using EIA.

**Results:** Akt activity (GSK-3 phosphorylation) was increased in ZDF12 as compared to ZL ($3.02 \pm 0.8$, $p<0.01$ vs. ZL). P-Akt expression followed a similar trend. Increases in Akt with age corresponded to increases in P-Ins. In STZ, the highest Akt activity was observed in STZ4 (STZ0: $2.1 \pm 0.4$; STZ4: $2.5 \pm 0.4$; STZ12: $1.4 \pm 0.4$ $p<0.01$ STZ4 vs. C). In contrast to Akt activity assay, expression of P-Ser-Akt was reduced in STZ0, whereas in other groups P-Akt was similar as in C (STZ0: $0.46 \pm 0.04$; STZ4: $0.77 \pm 0.14$; STZ12: $0.90 \pm 0.16$, $p<0.01$ STZ0 vs. C, $p<0.01$ STZ12 vs. STZ0, $p<0.05$ STZ4 vs. STZ0). P-Akt expression corresponded to P-Ins. In both models of DM, total Akt expression was similar as in controls.

**Conclusions:** In parallel with the development of metabolic syndrome, ZDF demonstrated progressive increase in Akt activity. This finding suggests that renal cortex is not affected by insulin resistance in DM2. In DM1, the highest Akt activity was observed in rats with moderate hyperglycemia and insulin levels comparable to C (STZ4). Akt serine phosphorylation, reflecting insulin actions, was reduced in severely hyperglycemic with insulinopenia (STZ0). This suggests that in addition to insulin renal Akt activity in DM1 is modulated by other factors, such as hyperglycemia. Considering the functions of Akt, these findings may help to elucidate structural and hemodynamic abnormalities associated with early stages of nephropathy in DM.

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## 1107

### iNOS and AGEs modulate $VEGF_{165}$-induced angiogenesis in glomerular endothelial cells (GENC) through VEGFR-2

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**Background and aims:** Vascular endothelial growth factor (VEGF) is a potent cytokine that stimulates angiogenesis, and microvascular hyperpermeability, effects that are largely mediated by endothelial nitric oxide synthase (NOS). It has been suggested that increased production of NO may be responsible for increased blood flow in renal beds in early diabetes. The expression of VEGF is pronounced in glomerular cells and its biological effects are mediated by specific receptors but their function in renal physiology and pathophysiology is partially unknown. Therefore, the aim of our study has been to evaluate the expression, function and modulation of binding sites for VEGF with high affinity (VEGFR-2) and with lower affinity (heparan sulfate proteoglycans, HSPG) in GENC.

**Materials and methods:** Binding studies were performed using displacement curves with increasing concentrations of VEGF. Gene expression has been evaluated using semi-quantitative RT-PCR. [$^3$H]Thymidine incorporation was used to determine cell proliferation. NO-synthase has been quantified with a commercial kit evaluating the conversion of [$^3$H]L-arginine in [$^3$H]L-citrulline and NOS enzymes have been characterized by ImmunoWestern Blot.

**Results:** $VEGF_{165}$ and $VEGF_{121}$ (a VEGF analogous with no affinity for HSPG binding sites) stimulated at low doses (0.1–1nM) the NO production whereas only at much higher concentrations (10nM) $VEGF_{165}$ was able to increase [$^3$H]Thymidine incorporation. In presence of 1 nM $VEGF_{165}$ and $VEGF_{121}$ for 1 to 5 days GENC showed a significant peak of inducible NOS (iNOS) production in the first day and through all the times of the experiment. Endothelial NOS (eNOS), instead, was significantly increased only starting from the third day. The co-presence of $VEGF_{165}$ with aminoguanidine (3 mM), an iNOS inhibitor, determined a quick increase of eNOS in the first day and induced a significant increase on [$^3$H]Thymidine incorporation in GENC suggesting an inhibitor role played by iNOS both on growth and eNOS production . Advanced glycation end products (AGEs) have been involved in the pathogenesis of diabetic nephropathy but the link with the VEGF system in the early phase of diabetic nephropathy has not fully been elucidated. In our model, AGE 1 mg/ml up-regulated the expression of VEGFR-2 with a parallel decrease of low affinity binding sites. In addition, AGE were able to reduce GENC growth and stimulate iNOS $VEGF_{165}$ controlled activity.

**Conclusion:** This study demonstrates, for the first time, that in GENC VEGFR-2 is a mediator of nitric oxide (iNOS and eNOS) release under VEGF control whereas the low affinity binding sites seem to mediate the weak growth effect observed in addition to the anti-mitogenic action showed by iNOS. The presence of AGE, up-regulating the VEGFR-2 and decreasing the low affinity binding sites, might partecipate to the blockade of glomerular angiogenesis, addressing in this way the effect of VEGF on glomerular endothelial wall towards the dramatic increase of permeability, as observed in the early phase of diabetic nephropathy.

## 1108

### The MCP-1/CCR2 system in human mesangial cells: modulation by stretch and role in ICAM-1 expression

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**Background and aims:** Inflammatory processes are believed to contribute to the pathogenesis of the glomerular injury in diabetes. Both the chemokine Monocyte Chemoattractant Protein-1 (MCP-1) and the monocyte adhesion molecule ICAM-1 are overexpressed in the glomeruli from diabetic animals and diabetic ICAM-1-deficient mice are resistant to renal injury. In vitro studies have shown that both high glucose and mechanical stretch, mimicking the glomerular insult of glomerular capillary hypertension, are potent MCP-1 inducers in mesangial cells. In addition, human mesangial cells (HMC) can express the MCP-1 receptor, CCR2, raising the hypothesis that MCP-1 has direct pro-inflammatory effects in this cell type. We investigated in HMC whether 1) mechanical stretch alters CCR2 expression; 2) MCP-1 increases cell-surface ICAM-1 protein expression leading to enhanced monocyte adhesion.

**Materials and methods:** HMC, serum-deprived for 24 hours, were exposed to 1) mechanical stretch (10% elongation – Stress Unit) 2) rh-MCP-1 (5-50-100 ng/ml) or vehicle for 3, 6, 12, and 24 hours. CCR2 mRNA and protein levels were measured by RT-PCR (primers - Genbank NM000648) and western blotting using a specific anti-human CCR2 antibody. ICAM-1 expression on mesangial cell surface was assessed by indirect immunofluorescence using a mouse anti-human ICAM-1 antibody. Both mesangial cells pre-exposed for 24 hours to MCP-1 (10 ng/ml) and control cells were co-incubated for 15 hours with human monocytes labelled with a fluorescent dye; adherent monocytes were then counted under contrast microscopy.

**Results:** Exposure to stretch induced a significant reduction in CCR2 mRNA levels at 3, 6, and 12 hours with a return to the baseline by 24 hours (3 hours: 29%, 6 hours: 49%, and 12 hours 45%, percentage reduction, $p<0.05$ stretch vs non-stretch). HMC exposure to MCP-1 induced a significant increase in ICAM-1 protein expression at 24 hours with no significant changes at earlier time points (3 hours: 0.93; 6 hours: 1.02; 12 hours: 0.99; 24 hours: 4.24 fold increase over control; $p<0.01$ 24 hours-exposure to MCP-1 vs vehicle). This effect was seen at all concentrations and was maximum at 5 ng/ml (5 ng/ml: 4.06; 50 ng/ml: 3.62; 100 ng/ml: 3.92 fold increase over control, $p<0.01$ MCP-1 vs vehicle). Mesangial cell pre-exposure to MCP-1 induced a significant 2.7-fold increase in monocyte adhesion as compared to control.

**Conclusion:** In conclusion stretch, a known MCP-1 inducer in HMC, down-regulates CCR2 expression and may thus enhance MCP-1 bioavailability and monocyte recruitment. The observation that MCP-1 induces ICAM-1 expression and monocyte adhesion to HMC indicates that the MCP-1/CCR2 system, besides its chemotactic property, has direct proinflammatory effects in HMC.

# PS 112

# Nephropathy: experimental pathophysiology and intervention

## 1109

**Glomerular number may influence the rate of progression of diabetic glomerulopathy**

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**Background and aims:** Individuals of low birth weight have a reduced number of glomeruli and thus may be at increased risk of glomerulosclerosis if they develop diabetes. Damage to the podocyte may be instrumental in the progression of renal disease. The aim of this study was to determine whether glomerular number has any influence on the glomerular structural changes associated with diabetes.
**Materials and methods:** Female offspring from Wistar rats fed normal (NPD) or low protein diet (LPD) in utero were studied. At 12 weeks of age, animals were randomised to control or diabetic groups. Diabetes was induced by streptozotocin injection (55 mg/kg body weight). After 28 weeks of diabetes, body weight, 24 h urine albumin excretion (UAER) and creatinine clearance (CrCl) were measured.
After perfusion fixation, the right kidney was removed and weighed.
Tissue was processed for light and electron microscopy. Structural parameters were estimated using standard stereological techniques on 12 diabetic and 12 control animals.
**Results:** Glomerular number (GN) was lower in LPD compared to NPD animals (27187 ± 3197 v 33882 ± 4580, p = 0.001).
Both groups of diabetic animals showed increased CrCl (LPD: 2.3 ± 0.5 v 1.5 ± 0.5 ml/min, p = 0.034; NPD: 2.7 ± 0.6 v 1.7 ± 0.3 ml/min, p = 0.011) and UAER (LPD: 32.9 (11.8–99.2) v 3.5 (1.1–49.2) mg/24 hr), p = 0.014; NPD: 15.8 (5.5–47.9) v 4.9 (0.5–9.9) mg/24 hr, p = 0.016) compared to corresponding control animals.
Both groups of diabetic animals had increased MGV, and glomerular basement membrane width compared to corresponding control groups. There was a significant negative correlation between mean glomerular volume (MGV) and GN in control animals (r = –0.64, p = 0.035), but not in diabetic animals (r = –0.38, p > 0.05).
In the diabetic animals (NPD plus LPD), lower GN was associated with increased foot process width on the mesangio-urinary surface (r = –0.72, p = 0.008). There was no such relationship in the control animals (r = –0.47, p > 0.05).
Although podocyte number was not significantly different between the two groups of diabetic animals (136 ± 43 v 173 ± 25, p = 0.095), there was a significant difference in podocyte density per glomerulus (85 ± 23 v 130 ± 25 /$10^6 \mu m^3$, p = 0.010) in LPD diabetic compared to NPD diabetic animals.
**Conclusion:** A reduced number of glomeruli results in compensatory glomerular hypertrophy. If diabetes develops in an animal with low glomerular number, the resulting structural and haemodynamic changes in an already enlarged glomerulus may subject the podocyte to mechanical stress earlier in the disease. This may accelerate podocyte damage and the eventual development of glomerulosclerosis.
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## 1110

**RAGE and TGFβ receptor-mediated signals converge on STAT5 and $p21^{WAF}$ to control cell-cycle progression of mesangial cells: a possible role in the development and progression of diabetic nephropathy.**

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**Background and aims:** The renal structural alterations in diabetic patients, prone to diabetic nephropathy, are characterized on early appearance of hypertrophy in both glomerular and tubular components, and by a late extracellular matrix accumulation leading to thickened glomerular and tubular basement membrane and progressive increase in mesangial mass. Mesangial cell (MC) hypertrophy in response to excess of glucose seems to be mediated by specific intracellular signal transduction mechanisms mainly regulating cell-cycle events, which are prevalently due to the release of several growth factors, including the transforming growth factor β (TGFβ). Moreover, besides the direct effects of hyperglycemia, recent data suggest an emerging role for the advanced glycation end products (AGE) in the pathogenesis of diabetic complications. The aim of the present study was to investigate the molecular events associated with acute and chronic exposure of MC to hyperglycemia or to TGFβ and in particular the molecular mechanisms regulating MC growth.
**Materials and methods:** We analysed cell-cycle events as well as the expression of $p21^{waf}$ following high glucose (HG), Amadori adducts, AGE and TGFβ treatment both alone or in combination with neutralizing antibodies against TGFβ and the receptor for AGE (RAGE). Moreover, the activation of the transcriptional factor STAT5 and its possible role in regulating either the expression of the cell-cycle-associated protein $p21^{waf}$ or the production of extracellular matrix proteins in response to these stimuli have been also evaluated. For this study MC ectopically transfected with an inactive or a constitutive active STAT5 mutants and $p21^{waf}$ –/– fibroblasts were used. Kidney biopsies from early and advanced stages of diabetic nephropathy were also analysed by immunofluorescence.
**Results:** We found that, unlike high glucose (HG) and Amadori adducts, AGE and transforming growth factor β (TGFβ) induced $p21^{waf}$ expression and accumulation of MC in G0/G1. TGFβ1 blockade inhibited AGE-mediated collagen production, but only partially affected AGE-induced $p21^{waf}$ expression and cell-cycle events, indicating that AGE by binding to AGE receptor (RAGE) „*per se*" could control MC growth. Moreover, AGE and TGFβ treatment led to the activation of the signal transduction and activators of transcription (STAT)5 and the formation of a STAT5/p21SIE2 complex. The role of STAT5 in AGE- and TGFβ-mediated $p21^{waf}$ expression and growth arrest, but not collagen production, was confirmed by the expression of the dominant negative STAT5 (ΔSTAT5) or the constitutively activated STAT5 (1*6-STAT5) constructs. Finally, in $p21^{waf}$-/- fibroblasts both AGE and TGFβ failed to inhibit cell-cycle progression. A potential in vivo role of these mechanisms was sustained by the increasing immunoreactivity for the activated STAT5 and $p21^{waf}$ in kidney biopsies from early to advanced stage of diabetic nephropathy.
**Conclusion:** Our data indicate that AGE- and TGFβ- mediated signals, by converging on STAT5 activation and $p21^{waf}$ expression, may regulate MC growth.
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## 1111

**Methotrexate prevents renal injuries through its anti-inflammatory effects in experimental diabetic rats.**

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**Background and aims:** Several mechanisms, such as glycation, oxidative stress, activation of protein kinase C, and hemodynamic change are believed to contribute to the progression of diabetic nephropathy. In addition to these mechanisms, we have recently shown that inflammatory mechanisms are involved in the pathogenesis of diabetic nephropathy using ICAM-1 deficient mice (Diabetes 52, 2003). Methotrexate (MTX), a folate antagonist, is widely used for the treatment of inflammatory diseases. Recently, it has been reported that treatment with low-dose MTX therapy reduces the risk of crdiovascular death in the patients with rheumatoid arthritis, suggesting that MTX exerts anti-atherosclerotic effects through anti-inflammatory actions. In the present study, we evaluated the effects of MTX on experimental diabetic nephropathy.
**Materials and methods:** Four-week-old male Sprague-Dawley (SD) rats were used. Diabetes was induced by intravenous injection of streptozotocin (STZ) at a dose of 65 mg/kg. MTX was dissolved in PBS and intraperitoneally injected once a week. The rats were randomly divided into five groups of 12 rats each : (a) non-diabetic control rats (Control), (b) diabetic rats received vehicle alone (DM), (c) diabetic rats administered with MTX at a dose of 0.5 mg/kg/week starting at 1 week after STZ injection (MTX0.5), (d) diabetic rats administered with MTX at a dose of 1.0 mg / kg /week starting 1 week after STZ injection (MTX1.0), and (e) diabetic rats that were administered with MTX at a dose of 1.0 mg/kg/week starting on the day prior to the STZ injection (preMTX). At 1, 4 and 8 weeks after induction of diabetes, body weight, systolic blood pressure (SBP), hemoglobin A1 c (HbA1 c), serum creatinine, urinary albumin excretion (UAE) and creatinine clearance were measured. At 8 weeks, rats were killed under anesthesia, and kidneys were harvested. Glomerular size and mesangial matrix area were measured by morphometry. We evaluated the number of macrophages and expression of ICAM-1 in glomeruli by immunohistochemistry. We also evaluated the activation of nuclear factor-κ B (NF-κB) by electrophoretic mobility shift assay and quantified gene expression of TGF-β1 by real-time RT-PCR in renal cortex.

**Results:** There was no significant difference in blood glucose level, HbA1 c, SBP and body weight in DM group and MTX treatment groups. UAE was increased in DM group and significantly reduced in MTX treatment groups in a dose dependent manner (Control; 221 ± 55, DM; 1123 ± 201, MTX(0.5); 629 ± 98*, MTX(1.0); 386 ± 59*, preMTX; 377 ± 39* μg/day, *p<0.05 vs. DM). Infiltration of macrophage and expression of ICAM-1 in glomeruli were increased in DM group and dose-dependently decreased in MTX treatment groups. NF-κB activity and TGF-β1 mRNA of renal cortex were also increased in the DM group and reduced in MTX treatment groups. Mesangial matrix expansion was also significantly suppressed by MTX.
**Conclusion:** Treatment with MTX ameliorated renal injuries without changes of blood glucose level and blood pressure in experimental diabetic rats. MTX may exert reno-protective effects by the anti-inflammatory actions through inhibition of NF-κB activation, ICAM-1 expression and macrophage infiltration. Inflammatory process might be a therapeutic target for diabetic nephropathy.

## 1112

**Thiazolidinedione ameliorates renal injuries through anti-inflammatory actions by inhibition of NF-κB activation in experimental diabetic rats**

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**Background and aims:** Thiazolidinedione (TZD), a ligand for peroxisome proliferator-activated receptor-γ (PPAR-γ), has been shown to reduce the progression of atherosclerosis through its anti-inflammatory actions. We previously reported that intercellular adhesion molecule-1 (ICAM-1) is up-regulated and promotes macrophage infiltration in the renal glomeruli and interstitium in human and experimental diabetic nephropathy. Furthermore, we have shown that ICAM-1 deficient mice are protected from renal injuries after induction of diabetes suggesting that inflammatory process is one of the critical factors for development of diabetic nephropathy. Recently, TZD has been reported to exert reno-protective effects in diabetic animals, although it remains unclear whether anti-inflammatory actions are involved in this process or not. The present study is designed to test the hypothesis that TZD prevents the progression of diabetic nephropathy by modulating inflammatory processes independent of insulin sensitizing effect.
**Materials and methods:** Male Sprague-Dawley rats aged 5weeks were used. Diabetes was induced by injection with streptozotocin (65 mg/kg body weight). We divided the rats into three groups: (i) non-diabetic control rats (non-DM) (n=6); (ii) diabetic rats (DM) (n=12) and (iii) diabetic rats treated with pioglitazone (DM+pio) (n=6). The DM+pio group received 0.0002% pioglitazone mixed in chow from a day before STZ injection. The non-DM and DM groups received normal chow. Blood glucose, hemoglobin A1 c (HbA1 c), systolic blood pressure, serum creatinine, urinary albumin excretion (UAE), creatinine clearance, and body weight were measured at 1, 4, and 8 weeks after induction of diabetes. Rats were killed at 8 weeks under anesthesia and the kidneys were harvested. We measured glomerular size by morphometry and examined glomerular macrophage infiltration and ICAM-1 expression by immunohistochemistry. We also analyzed the activation of nuclear factor-kappa B (NF-κB), which is the major regulator of ICAM-1, using electrophoretic mobility shift assay.
**Results:** Blood glucose, HbA1 c and systolic blood pressure were elevated in diabetic rats, but not changed by treatment with pioglotazone. UAE was increased in DM group (693±106μg/day ) as compared with non-DM group (213±42 μg / day) and significantly decreased in DM+pio group (272 ± 52 μg / day, p<0.05 vs. DM group). Pioglitazone also suppressed glomerular hypertrophy in diabetic rats. Immunohistochemistry revealed that expression of ICAM-1 and infiltration of macrophage in glomeruli and interstitium were increased in diabetic rats and significantly reduced by pioglitazone. Moreover, renal NF-κB activity was increased in diabetic rats and suppressed by pioglitazone.
**Conclusion:** Pioglitazone prevented renal injuries without change of blood glucose levels and systemic blood pressure in experimental diabetic rats. The reno-protective effects of pioglitazone may be mediated by anti-inflammatory actions including the inhibition of NF-κB activity, ICAM-1 expression and macrophage infiltration. TZD might exert protective effects against diabetic nephropathy through anti-inflammatory actions.

## 1113

**Sorbitol dehydrogenase inhibition as well as aldose reductase inhibition prevents elevation of urinary albumin excretion in diabetic rats**

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**Background and aims:** Microalbuminuria is an important marker and risk factor for progression of diabetic nephropathy. However, the biochemical mechanisms that initiate diabetic albuminuria are poorly understood. Previous data with aldose reductase inhibitors (ARIs) imply that elevated metabolic flux through aldose reductase (AR), the first enzyme of the polyol pathway (PP), plays an important role in the onset and maintenance of diabetic albuminuria. However, there are no data as to whether metabolic flux through the second enzyme of the PP, sorbitol dehydrogenase (SDH), is linked to development of albuminuria. To address this question, we characterized the *in vitro* and *in vivo* properties of a sorbitol dehydrogenase inhibitor (SDI) and compared the effect of the SDI vs. two structurally distinct ARIs on the development of elevated urinary albumin output (UAO) in diabetic (Db) rats.
**Materials and methods:** *In vitro* studies of SDI CP-642931 (S1), ARIs zopolrestat (A1) and CP-744809 (A2) employed standard enzymatic methods. *In vivo* pharmacological activity was tested in groups of 5 male streptozocin (STZ)-Db rats (85 mg/kg, i.v.). 7 days post-STZ rats were dosed orally q.d. for 5 more days with vehicle or S1, A1 or A2. 4 hrs after the last dose, rats were euthanized and kidneys dissected, weighed and assayed for fructose and sorbitol by standard methods. In a 4-week UAO study using a Balanced Randomized Complete Block design, groups of 30 STZ-Db rats were administered feed either plain or containing S1 to give ~2 mg S1/kg BW/d or A1 or A2 at ~60 mg A1 or A2/kg BW/d. At 4 weeks 24 hr urine was collected in a metabolic cage for each rat and analyzed for UAO using Nephrat kits (Exocell, Philadelphia). UAO data were log transformed and analyzed with SAS Version 8; other data are mean ± SD (n).
**Results:** S1 inhibited rat and human SDH *in vitro* with Ki = 0.20 ± 0.07 (6) nM. S1 was a highly specific inhibitor of SDH: S1's IC50s for AR, alcohol-, fructose- and lactate- dehydrogenases were >3500-fold > S1's IC50 for SDH. I*n vitro* Kd for ARIs A1 and A2 vs. human AR = 3.3 ± 0.2 (3) nM and 0.53 ± 0.28 (3) nM, respectively; both ARIs were highly specific for AR. Oral S1 for 5 days at 3–20 mg/kg BW/d dose-dependently normalized Db-elevated renal cortical fructose 72–88%, comparable to 100 mg/kg BW/d A1, 71%, and 10–60 mg/kg BW/d A2, 83–87% (all P<0.05 vs. Db). Cortical sorbitol values were unaffected by S1 (–15 to +17%, NS), while A1 normalized sorbitol by 55% and A2 by 55–77% (all P<0.05). In the 4-week study UAO (mg/d) in normal rats was 0.8 (0.5–1.2, 95% C.I.) vs. 2.0 (1.4–2.7) in Db rats, an elevation of 2.5-fold (P<0.05); S1 normalized UAO in Db rats by 66%, 1.2 (0.9–1.6) (P=0.023 vs. Db); A1 by 64%, 1.2 (0.9–1.7) (P=0.043); and A2 by 91%, 0.9 (0.7–1.2) (P=0.0005). Urinary volume and blood glucose were not affected by any of the treatments.
**Conclusions:** These data provide further evidence that elevated metabolic flux through the PP plays an important role in the pathogenesis of diabetic albuminuria. They also point, for the first time, to the importance of metabolic flux through the second PP enzyme, SDH, in the pathogenesis of diabetic albuminuria.

## 1114

**Tight blood pressure control corrects renal abnormalities in experimental diabetes mellitus**

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**Background and aims:** The aim of this study was to examine the effect of the prevention of arterial hypertension with: captopril (CA), triple therapy (hydralazine, reserpine and hydrochlorothiazide) (TRI) or losartan (LOS) over the renal cell replication, albuminuria and accumulation of renal fibronectin (FN) in diabetic spontaneously hypertensive rats (SHR).
**Materials and methods:** Diabetes Mellitus (DM) was induced by injecting streptozotocin (60 mg/kg) in SHR rats and their normotensive control Wistar-Kyoto (WKY) at 4 weeks of age. The rats were killed 20 days after the induction of diabetes. Blood glucose concentration and systolic blood pressure (SBP) were measured before sacrifice. Albumin excretion rate (AER) was determined by radial immunodifusion, replicating renal cells were estimated with immunohistochemistry (proliferating cell nuclear

antigen) (PCNA) and renal fibronectin was estimated by imunofluorescence (IMF) and Western Blot analysis (WB).
**Results:** The results are expressed as means±SD except for AER wich are expressed as geometric mean (range).

| Groups | Body Weight (g) | SBP (mmHg) | Glicemia (mg/dl) | AER (mg/24h) | FN (densit u) | FN (IMF) | PCNA (cel/glom) |
|---|---|---|---|---|---|---|---|
| WKY C | 225±15 | 125±10 | 141±34 | 84 (62–141) | 0.70±0.75 | 1.26±0.191 | 2.93±1.00 |
| WKYD | 161±33$^{\Delta}$ | 127±23 | 521±44$^{\Delta}$ | 263(78–1033)$^{\Delta}$ | 0.77±0.64 | 1.84±0.416 | 1.21±0.69$^{\Delta}$ |
| SHR C | 165±20 | 155±6 | 117±28 | 132 (57–717) | 2.27±2.15 | 1.69±0.330$^{\S}$ | 3.68±2.34$^{\S}$ |
| SHR D | 86±18$^{\S}$ | 145±16* | 488±62$^{\S}$ | 372(111–501)$^{\S}$ | 7.61±1.22$^{\S}$* | 3.23±0.534* | 1.64±0.85 |
| CA | 99±25 | 118±15$^{\epsilon}$ | 506±114$^{\epsilon}$ | 153 (63–543)$^{\epsilon}$ | 2.49±1.42$^{\epsilon}$ | 2.22±0.620$^{\epsilon}$ | 3.89±1.60$^{\epsilon}$ |
| LOS | 116±14 | 111±9$^{\epsilon}$ | 524±61$^{\epsilon}$ | 146 (61–381)$^{\epsilon}$ | 1.57±.1.1$^{\epsilon}$ | 1.54±0.487$^{\epsilon}$ | 3.77±1.31$^{\epsilon}$ |
| TRI | 91±8 | 112±14$^{\epsilon}$ | 509±67$^{\epsilon}$ | 176 (68–449)$^{\epsilon}$ | 2.04±1.42$^{\epsilon}$ | 1.92±0.487$^{\epsilon}$ | 3.93±0.48$^{\epsilon}$ |

P<0.05 $^{\S}$ SD × SC; *SD x WD; $^{\epsilon}$ treated groups × SD; $^{\Delta}$ WKY D × WKY C

**Conclusion:** In diabetic SHR rats tight blood pressure control, independently of the class of antihypertensive agent, restores the renal cellular replication and prevents the increment in albumin excretion rate and renal fibronectin.
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## 1115

**Antioxidants, ferulic acid and alpha-tocopherol, may prevent the pathological and functional abnormalities of the kidney in OLETF-diabetic rats**
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**Background and aims:** Our aim was to examin the preventive effect of ferulic acid (polyphenol included in rice bran) and alpha-tocopherol on the neuropathy and nephropathy in spontaneous diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats.
**Materials and methods:** We used OLETF rats as a model of type 2 diabetes mellitus with obesity, and non-diabetic Long-Evans Tokushima Otsuka (LETO) rats as a control. The OLETF rats were divided into three groups (OLETF rats with a usual diet: the DM group, with a diet including 0.2% ferulic acid: the FA group, and 0.5% alpha-tocopherol: the AT group). The LETO rats (the CON group) were bred with a usual diet. All of the OLETF rats were diagnosed as diabetic by the criterion of blood glucose >250 mg/dL. Twelve weeks after the diagnosis of diabetes, the nerve conduction velocities (NCV) of the sciatic-tibial nerve in all of the rats were measured under the pentobarbital anesthesia. The rats were sacrificed for the collection of their blood and kidneys. Plasma glucose, (PG) glycated hemoglobin (GHb), urinary 8-isoprostane and 8-hydroxy deoxyguannosine (8-OHdG) were measured. Furthermore, the mRNA expressions of antioxidant enzymes, Cu-Zn superoxide dismutase (SOD) and glutathione peroxidase 1 (GPx), and the adhesion molecules, soluble intercellular adhesion molecule 1 (ICAM) and soluble vascular adhesion molecule 1 (VCAM) in the kidneys were quantified using a real-time polymerase chain reaction method. The kidneys were fixed by formaldehyde, and stained with HE and PAS, then histopathologically evaluated. Pathological change was quantified as a pathology index by scoring the amount of PAS positive extracellular matrix deposited in the mesangial region of the glomeruli.
**Results:** PG, GHb, 8-isoprostane and 8-OHdG levels were elevated in the DM, FA and AT groups compared to the CON group, but the difference among the three diabetic groups was not significant. NCV in the DM, FA and AT groups was significantly lower than in the CON group, however there were no differences among the three diabetic groups. Messenger RNA expression of SOD, GPx, ICAM and VCAM in the kidney did not changed significantly by the administration of FA or AT. The urinary protein excretion level in the DM group increased significantly compared to the CON group. By the administration of FA and AT, the increase in urinary protein was significantly suppressed. There was a significant increase of glomerular lesions in the diabetic rats. The severity of the glomerular lesions was improved by the administration of FA or AT.
**Conclusion:** These findings suggest the possibility that antioxidant supplementations have a beneficial effect on the prevention of diabetic nephropathy.

# PS 113
# Kidney function; pancreas and renal transplantation

## 1116

**Renal function among elderly diabetic subjects: Assessment by serum cystatin C, serum creatinine and the urinary albumine-to-creatinine ratio**
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**Background and aims:** Diabetic nephropathy is increasingly becoming a disease of older people, but our understanding of diabetic nephropathy is primarily based on research done among middle-aged populations, and direct extrapolation of these results to the elderly, especially to the very old, may be inappropriate. The objectives of the study were to characterize renal impairment associated with diabetes in older adults by means of serum markers of glomerular filtration rate and microalbuminuria tests and, especially, to evaluate possible differences between older people below 80 years of age and the very old.
**Materials and methods:** Renal function was estimated by serum Cystatin C, serum creatinine, and the urinary albumine-to-creatinine ratio in 187 diabetic and 1,073 non-diabetic subjects (age range 65–100 years) participating in a cross-sectional population-based survey in southwestern Finland. Diabetes was defined based on the medical history or fasting plasma glucose concentrations of 7 mmol/L or higher. Determinants of elevated levels of serum Cystatin C and serum creatinine and microalbuminuria were assessed by multivariate analysis.
**Results:** In the very old ( ≥80 years), diabetes, after adjustment for confounders, was significantly associated with elevated levels of serum Cystatin C (P = 0.011) and serum creatinine (P = 0.004). In the younger age group (<80 years), diabetes was not independently associated with elevated levels of serum Cystatin C or serum creatinine, whereas the impact of hypertension was highly significant (P < 0.001). In the older group, hypertensive diabetic subjects, compared to diabetic subjects without hypertension, had lower mean levels of serum Cystatin C (1.47 mg/L ± 0.55 vs. 1.62 mg/L ± 0.71) and serum creatinine (109.4µmol/L ± 26.2 vs. 115.0µmol/L ± 45.0). The prevalence of microalbuminuria among diabetic subjects was 29.7%, and 15% had elevated serum creatinine levels (reference limit; men 118µmol/L, women 104µmol/L), whereas the prevalence of elevated serum Cystatin C levels varied considerably depending on whether adult (0.95 mg/L) or age-adjusted regression-based reference limits were used (64.7% vs. 21.4%). 64.1% of diabetic subjects with elevated serum Cystatin C levels and 48.2% of subjects with elevated serum creatinine levels did not have microalbuminuria.
**Conclusion:** The impact of diabetes on renal impairment changes with increasing age, probably reflecting the combined impact of selective survival and age-related changes in the pathophysiology of diabetes. Serum markers of glomerular filtration rate and microalbuminuria identify renal impairment in different segments of the diabetic population, indicating that serum markers as well as microalbuminuria tests should be used in screening diabetic older people for kidney damage. The appropriate reference limit for serum Cystatin C in geriatric clinical practice must be defined by further research.

## 1117

**The Cockcroft and Gault formula in diabetic subjects: Biases and their correction**
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**Background and aims:** The Cockcroft and Gault formula (CG) is the recommended basis of the evaluation of renal function in diabetic patients, but it may be biased at extreme Glomerular Filtration Rate (GFR), Body Mass Index (BMI) or age. Our aim was to determine these biases, and to correct them.
**Materials and methods:**153 non-dialyzed diabetic subjects (85 men/68 women ; 46 type 1 diabetes/107 type 2) with a mean HbA1C of 8.6 ± 1.6% and a mean serum creatinine level of 136.5 ± 70.5 µmol/L (54–371) were

studied. A wide range of GFR (8–196 mL/min ; mean : 63.9 ± 41.3 mL/min), BMI (15.6–48.9; mean : 27.5 ± 4.8), and age (19–83 years old; mean : 62.1 ± 13.8 yrs) were represented. We compared CG to GFR measured by an isotopic method (51Cr-EDTA) by correlation, paired t tests, and a Bland & Altman procedure (B&A). Comparisons were repeated after categorizing the subjects in tertiles of GFR, BMI and age. Biases were considered as significant if $p<0.05$ between paired values of isotopic GFR and CG, and then expressed as %GFR. The CG formula was then modified, by introduction of height2 and deletion of weight at the numerator, and exponential expression of serum creatinine at the denominator. Combination of these modifications led to a new formula: (1.2 X (height [m])2 X (140-age [yrs]) X K)/exp(creatinine[μM]/70) + 5, with K=1.23 for men and 1.04 for women, that was also tested against isotopic GFR.
**Results:** Overall mean CG and GFR were similar (CG: 60.5 ± 30.9, GFR: 63.9 ± 41.3 mL/min; NS), and well correlated ($r=0.83$, $p<0.0001$), but the CG was significantly biased as shown by the B&A procedure : CG less GFR were negatively correlated to their means ($r=0.471$, $p<0.001$). Analysis by tertile confirmed the biases according to GFR (50% overestimation at low GFR and –18% underestimation at high GFR). It also detected a –15% underestimation at the lowest tertile of BMI (no bias according to age). Introduction of height2 and deletion of weight corrected the bias at low BMI. Exponential expression of serum creatinine corrected the bias at extreme values of GFR. The new formula was well correlated to isotopic GFR ($r=0.84$, $p<0.0001$), and not biased for GFR, BMI, and age. By contrast with the CG formula (lowest value: 15.7 mL/min), this new formula allowed the diagnosis of terminal renal failure (GFR<15 mL/min) with a 77% sensiblility and a 93% specificity. The sensibility for the diagnosis of severe renal failure (GFR<30 mL/min ; sensibility : 77% vs 51% for CG) and the specificity for the diagnosis of moderate renal failure (GFR<=60 mL/min ; specificity : 87% vs 72% for CG) were improved. The overall accuracy of the estimation was also improved (69% estimations within ± 30% of isotopic GFR vs 61% by CG).
**Conclusion:** The CG formula is biased at extreme GFR, and it underestimates GFR in diabetic subjects with low or normal BMI. These biases can be corrected, allowing an improvement of the diagnostic performances.

## 1118

**Post-transplant diabetes mellitus - risk factors, frequency of transplant rejections and long-term prognosis**

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**Background and aims:** Estimations about the incidence of new-onset diabetes (DM) after renal transplantation varying between 2 and 54%. It was the aim of the trial to stuy the prevalence of post-transplant DM, risk factors, the frequency of transplant rejections and long-term prognosis.
**Materials and methods:** All consecutive patients (n=253, age 52.2 ± 12.6 ys, BMI 22.0 ± 7.9 kg/m$^2$) with end-stage renal disease, but without DM were studied, receiving kidney transplantation at our centre since 1992 (follow-up 3.3 ± 1.6 [0.1–17.7] ys).
**Results:** In total 43/253 patients (17%) developed new-onset DM after transplantation. Patients with new-onset DM were significantly older (58.3 ± 11.4 vs 50.9 ± 12.5, $p<0.01$) and had a tendency to a higher BMI (24.0 ± 8.5 vs 21.6 ± 7.8 kg/m$^2$, $p=0.077$). There were no differences between the groups in respect of blood pressure control (137.7 ± 19.0/81.8 ± 14.2 vs 137.1 ± 21.9/83.9 ± 13.1 mmHg, p=0.89/0.39), glomerular filtration rate (58.0 ± 28.1 vs 64.1 ± 22.1 ml/min/1,73 m$^2$, $p=0.13$), steroid dosage during (4.5 ± 1.2 [n=21] vs 4.6 ± 2.2 [n=135] mg/d, $p=0.13$) or the frequency and dosage of other immunosuppressive drugs (cyclosporine, tacrolimus, sirolimus) during the follow-up. Patients with new-onset DM had higher creatinine values (163.4 ± 67.9 vs 138.7 ± 59.5 μmol/l, $p=0.017$). Mean HbA1 c (Tosho, mean normal 5.15%) of patients with DM was 6.28 ± 1.29%. In 18 patients (7,1%) transplant rejections occured (patients without DM 16 [7.6%] vs patiens with new-onset DM 2 [4.7%], $p=0.39$). Performing multivariate analysis the only parameter associated with new-onset DM was BMI (R-square=0.05, $\beta=0.23$, $p=0.02$), the only factor associated with transplant rejection was fasting blood glucose (R-square=0.07, $\beta=0.28$, $p=0.02$). All other parameters included in the models (age, duration after transplantation, diabetes duration, immunosuppressive therapy, HbA1 c, HLA-mismatches) showed no associations.
**Conclusion:** The prevalence of new-onset DM after renal transplantation was 17%. The most important parameter associated with new-onset DM was higher BMI, the most important parameter associated with transplant rejection were elevated fasting blood glucose levels. To prevent transplant rejections and to improve patients' outcome additional to an optimal immunosuppressive therapy and HLA-matching, good blood pressure control and HbA1 c, but also near norma fasting blood glucose levels should be achieved.

## 1119

**Lack of detrimental effect on renal function in diabetic renal transplant patients. Experience from the ALERT study. On behalf of the ALERT investigators**

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**Background:** Lately some concerns has been raised associated with a potential harmful effect of statins on renal function. Patients with diabetes mellitus and impaired renal function may be will suited to assess the long term renal effects of statin treatment.
**Methods/Patients:** 2102 stable renal transplant patients (total cholesterol 4.0–9 umol/l) were randomly assigned to treatment with fluvastatin 80 mg (n =1050) or placebo (n=1052) and followed for 5–6 years. The mean age was 50 years (30–75), 66% were male. Patients were classified as diabetic based on medical history recorded in the case record form. A distinction was made between insulin and non insulin depend patients. Overall 396 (18.8%) patients were identified as having diabetes, 261 male, 197 (18.8%) in the fluvastatin arm and 199 (18.9%) in the placebo arm . We evaluated the renal function evolution along the whole follow-up period in fluvastatin diabetics treated patients as compared to placebo, by assessing creatinine, creatinine clearance and proteinuria.
**Results:** Overall the average serum creatinine at inclusion was 147 umol/l (range 70– 300). In the diabetic population , along the study the mean creatinine was 169.08 umol/l for the fluvastatin group and 165.02 umol/L in the placebo group (p = 0.4124) , the creatinine clearance in the fluvastatin group was 54.09 ml/min, as compared to 55.24 ml/min in the placebo arm (p = 0.2347). Proteinuria was 0.76 g/24 hours in the fluvastatin group and 0.72 g/24 hours in the placebo group p = 0.7422.
**Conclusions:** The ALERT demonstrated that fluvastatin can be safely administered and has no deleterious effect on renal function during long term follow up in diabetics renal transplant recipient.
*Supported by: Novartis Pharma AG*

## 1120

**Long-term prognosis of patients after kidney transplantation with versus without diabetes mellitus**

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**Background and aims:** In comparison to non-diabetic subjects, patients with type 2 diabetes (DM) and end-stage renal disease (ESRD) were seldom selected for renal transplantation. Thus, it was the aim of the trial to study the long-term prognosis after transplantation in patients with versus without DM.
**Materials and methods:** All consecutive patients (n=333) were studied, received a kidney transplant at our centre since 1992. Mean follow-up in 302/333 patients (91%) was 3.29 ± 1.54 (0.04–17.66) years. At the time of transplantation DM (type 1/2 n=3/46) was known in 49 patients.
**Results:** Patients with DM were older (patients without DM [n=253] vs patients with DM [n=49]: 52.2 ± 12.6 vs 58.8 ± 13.1, $p=0,002$), but they had a very good quality of diabetes control (HbA1 c, Tosho, mean normal NW 5.15%, patients with DM 6.33 ± 0.93% vs non-diabetic patients 5.15 ± 0.98%, $p=0.03$), and even, during the follow-up, patients showed a tendency to further improvement (patients with DM 5.67 ± 0.93% vs non-diabetic patients 5.46 ± 0.92%, $p=0.30$). At the end of the trial there were also no differences between the groups in respect of blood pressure control (patients with DM 135.3 ± 28.2/79.6 ± 17.2 vs non-diabetic patients 130.9 ± 28.7/78.8 ± 17.1 mmHg, p=0.33/0.78) and renal function (creatinine 142.9 ± 61.6 vs 151.8 ± 68.2 μmol/l, $p=0.38$, glomerular filtration rate: 63.1 ± 23.3 vs 59.1 ± 24.0 ml/min/1,73 m$^2$, $p=0.30$). In total 26 patients showed transplant rejections (patients with DM 8 [prevalence 16.3%] vs non-diabetic patients 18 [prevalence 7.1%], $p=0.11$). Performing multivariate analysis, the most important parameter associated with the incidence of

transplant rejections was fasting blood glucose (R-square=0.044, β=0.21, p=0.009). All other parameters included in the model (BMI, duration since transplantation, diabetes duration, immunosuppressive therapy, HbA1 c, HLA-mismatch) revealed no associations.
**Conclusion:** Following kidney transplantation the prevalence of rejections in patients with DM is slightly, but not significant higher compared to non-diabetic subjects. One of the most important risk factors seems to be fasting blood glucose. Hence, following renal transplantation treatment strategies should not only focused on optimal immunosuppressive therapy and HLA-matching, good HbA1 c and blood pressure control, but also on near normal fasting blood glucose levels.

## 1121

**Factors associated with early deaths on hemodialysis in diabetic patients. Romanian 9-year experience**
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**Background and aims:** Early death (in first 3 months) of the patients after their admission in renal replacement therapy programs is an important topic worldwide, because of its social, economic and medical related aspects. Our study aims to detect possible epidemiologic, clinic or metabolic factors associated with early death of diabetic patients on maintenance hemodialysis (HD).
**Materials and methods:** 198 diabetic patients (91F vs 117M; 90 T1DM vs 108 T2DM), admitted in the HD program in our centre between 1995–2003 were included in the study group, after the exclusion of those displaying incomplete data or technical drop-outs.Their recorded data were retrospectively analyzed.
**Results:** 60 patients (30%) died in the first 3 months after starting HD. The following distinctive features were observed in the dead patients group, compared with the survivors group (n=138): higher pre-dialysis session plasma bound urea nitrogen (BUN) concentrations (74.4 vs 64.6 mg/dl; p=0.02), probably as result of a catabolic state; lower hemoglobin (7.7 vs 8.6 g/dl; p= 0.0014) and hematocryte (23.5 vs 26,5; p= 0.0002) values, reflecting the importance of renal anemia severity for the prognosis of these patients; lower interdialytic weight gain (1.6 vs 2.2 kg; p=0.001), probably as result of poorer dietary intake; pre-dialytic session metabolic acidosis (pH 7.31 vs 7.35; p=0.001).
**Conclusion:** Factors associated in our study with death of diabetic patients with chronic renal failure, in the first 3 months after starting HD, are: catabolic state, metabolic acidosis, severe renal anemia and lower dietary intake. These data generally confirm the results of other studies in non-diabetic patients; nevertheless, the lower interdialytic weight gain, found in our study as a risk factor for early mortality, is an apparently surprising finding and we considered it as a marker of lower dietary intake. It is worthy, in our opinion, to focus on these factors, especially on the intensive treatment of renal anemia in the pre-dialysis period, in order to limit their deleterious effects on the life expectancy of these patients.

## 1122

**Cardiovascular risk factors (CRF) after kidney-pancreas transplantation (KPT)**
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**Background and aims:** Cardiovascular morbility and mortality in type 1 diabetic patient with end-stage renal disease can be reduced after KPT, although mechanism is not well known.
**Materials and methods:** We report on 9 type 1 diabetic patients (age: 37,3 ± 7 years; males/females: 4/5; BMI: 23,9 ± 2,1 Kg/m$^2$; duration of diabetes: 24,3 ± 5,8 years; immunosupression: tacrolimus, mycophenolatemophetil and steroids) who received KPT and were studied before transplantation and at 6 months. Before KPT all patients needed antihypertensive therapy and 8 out of 9 needed statins. Total cholesterol (TC), LDLc, HDLc, triglycerides (TG), apoprotein A (ApoA) and apoprotein B (ApoB) were determined with other CRF (systolic blood pressure [SBP], diastolic blood pressure [DBP] and body mass index [BMI]).
**Results:** (tables 1 and 2): Normalization of fasting plasma glucose and HbA1 c (p<0.007) with no exogenous insulin administration was achieved after KPT. Significant improvement of TG, SBP and DBP was also observed and BMI increased. TC, HDLc, LDLc, ApoA and ApoB were similar before and after transplantation. Moreover, after grafting, 8 out of 9 patients quit antihypertensive therapy and none need statins.

Table 1
Wilcoxon: (1) p<0,007; (2) p<0,01; (3) p< 0,05

| | Glucose | HbA1 c | SBP | DBP | BMI |
|---|---|---|---|---|---|
| Before TRP | 246 ± 89 | 8,4 ± 1,2 | 156 ± 7 | 96 ± 7 | 23 ± 2 |
| 6 month | 80 ± 8[1] | 4,7 ± 0,6[1] | 137 ± 15[2] | 79 ± 9[1] | 25 ± 3[3] |

Table 2
Wilcoxon: (1) p<0,007; (2) p<0,01; (3) p< 0,05

| | TC | LDLc | HDLc | TG |
|---|---|---|---|---|
| Before TRP | 189 ± 63 | 89 ± 47 | 63 ± 16 | 130 ± 51 |
| 6 month | 175 ± 37 | 99 ± 27 | 59 ± 12 | 88 ± 33[3] |

**Conclusion:** These results suggest KPT can early improve CRF such as HTA and TG and help to achieve an adequate lipid profile (TC, HDLc, LDLc and TG) without no need of statins.

## 1123

**Effects of pancreas transplant alone on diabetic nephropathy**
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**Background and aims:** Successful pancreas transplant alone (PTA) normalizes plasma glucose levels by restoring endogenous insulin secretion. The effects of PTA on diabetic nephropathy are still unsettled.
**Materials and methods:** In this study 23 type 1 diabetic patients (age: 39 ± 10 yrs; M/F: 12/11; BMI: 23 ± 3 Kg/m$^2$; duration of diabetes: 26 ± 10 yrs) with successful PTA (portal-enteric drainage) were evaluated before (preTx) and 1 year after transplantation (postTx). Maintainance immunosuppression was based on tacrolimus, MMF and low-dose steroids. Several clinical parameters were measured, including 24 h urinary protein excretion and creatinine clearance (by the Cockroft-Gault formula).
**Results:** Normoglycemia with insulin independency was maintained throughout the study period (postTx fasting plasma glucose, HbA1 c and fasting C-peptide concentrations were respectively: 85 ± 10 mg/dl, 5.3 ± 0.41%, and 2.9 ± 1.1 ng/ml). Creatinine levels remained unchanged (preTx and postTx values of 0.96 ± 0.29 and 1.01 ± 0.20 mg/dl, NS). Creatinine clearance was 90 ± 27 ml/min before transplantation and 84 ± 26 ml/min 1 year posttransplantation (NS), with no change of BMI. Urinary protein excretion decreased significantly from the preTx value of 1.5 ± 2.8 g/24 h to the postTx value of 0.8 ± 1.8 g/24 h (p<0.01). Sixty% of patients with microalbuminuria (30–300 mg/24 h) and 18% of those with overt proteinuria (>300 mg/24 h) became normoalbuminuric. Arterial blood pressure (systolic over diastolic) decreased significantly from 131 ± 14 over 82 ± 9 mmHg to 124 ± 10 over 76 ± 8 mmHg (p<0.05)
**Conclusion:** In conclusion, a significant decrease of urinary protein excretion without any change in creatinine levels or creatinine clearance occurred after PTA, showing that this procedure can have a positive impact on diabetic nephropathy; in this regard, both normalization of glucose levels and reduction of blood pressure values are likely to play a major role.

## 1124

**Islet auto-antibodies after pancreas transplantation**
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**Background and aims:** Autoimmune recurrence and subsequent diabetes after pancreas transplantation has been described. However sufficient immunosuppression might prevent recurrence of autoimmune diabetes.
**Materials and methods:** In this study 58 type 1 diabetic patients (age 38 ± 7 years, diabetes duration 27 ± 7 years) were examined 79 ± 48 months after successful pancreas/kidney transplantation (SPK). The prevalence of autoantibodies to glutamate decarboxylase (GAD) and tyrosine phosphatase (IA-2) as well as parameters of pancreas graft function (fasting blood glucose, oral glucose tolerance, HbA1 c and creatinine) were studied. According to immunoreactivity (GAD > 0.9 U / ml, IA-2 > 0.75 U / ml) graft recipients were grouped: group 1: no immunoreactivity, group 2:

immunoreactivity to one islet antigen; group 3: immunoreactivity to both antigens.
**Results:** 40 % of graft recipients displayed no immunoreactivity, 43 % showed positivity for one antigen and 17% were positive for both IA2 and GAD. There was no relation of immunoreactivity and time after transplantation. There were no significant differences concerning fasting glucose, HbA1 c, glucose tolerance and renal function between the three groups. Patients with ciclosporin (n= 30) as first line immunosuppression displayed more often immunoreactivity to IA2 than patients immunosuppressed with tacrolimus (n=28) (37 % versus 11%; p=0.02). Concerning GAD there were no significant differences between patients under ciclosporin or tacrolimus. Azathioprin or mycophenolat mofetil use was not associated with immunoreactivity.
**Conclusion:** Immunological markers for type 1 diabetes persist or reappear in more than the half of pancreas graft recipients despite heavy immunosuppression. Immunoreactivity to IA-2 was influenced by the type of calcineurin-inhibitor used. However immunoreactivity was not associated with impaired graft function.

## 1125

**Metabolic effect of tacrolimus versus cyclosporine on pancreatic graft function**
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**Background and Aims:** The diabetogenic effect of calcineurin inhibitors is generally known and is supposed to be higher in tacrolimus (Tacro) than in cyclosporine (Cyclo). The aim of our study was to compare the glucose metabolism in Type 1 diabetic recipients of kidney and pancreatic grafts on tacrolimus versus cyclosporine-based immunosuppression in conjunction with mycophenolate mofetil.
**Methods:** We examined 22 insulin-independent patients after simultaneous pancreas and kidney transplantation with systemic venous drainage of pancreatic graft (mean post-transplant period 2.2 ± 1.2 [SD] years). All recipients had a stabile good function of the kidney graft (mean serum creatinine level 110.9 ± 20.4 µmol/L). Fasting glycemia, insulin levels, glycosylated hemoglobin ($HbA_{1c}$), a standard intravenous glucose tolerance test (IVGTT) with coefficient of glucose assimilation ($K_G$) calculation and trough Tacro/Cyclo levels were assessed in pancreas recipients after elective steroid withdrawal (according to transplant protocol). Insulin sensitivity was evaluated 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:** Our groups (Tacro vs. Cyclo) did not differ in age, BMI and post-transplant period. We did not find any significant difference in response of IVGTT. In Tacro group (n = 11) 2 patients had an abnormal response to glucose stimulus ($K_G$ < 0.8 %/min.), 4 patients had an impaired glucose tolerance (0.8 ≤ $K_G$ < 1.2 %/min.) and 5 patients had a normal glucose tolerance ($K_G$ ≤ 1.2 %/min.). In Cyclo group (n = 11) the abnormal response was present in 1, the impaired glucose tolerance in 2 and the normal glucose tolerance in 8 recipients. The other results are shown in the following table.

**Table**

| | $HbA_{1c}$ IFCC (%) | Fasting glycemia (mmol/L) | HOMA-IR | $K_G$ (%/min.) | Total C-peptide secretion (pmol/mL) | Total insulin secretion (mIU/L) |
|---|---|---|---|---|---|---|
| Tacro group | 3.4 ± 0.4 | 4.75 ± 0.6 | 2.59 ± 1.58 | 1.26 ± 0.52 | 120.5 ± 49.3 | 1819 ± 899 |
| Cyclo group | 3.3 ± 0.4 | 4.57 ± 0.5 | 2.86 ± 2.12 | 1.46 ± 0.44 | 99.9 ± 33.9 | 1965 ± 1027 |
| Difference | NS | NS | NS | NS | NS | NS |

Trough levels of calcineurin inhibitors (Cyclo 134.5 ± 18.1 ng / mL; Tacro 10.1 ± 1.3 ng / mL) had no significant impact on any of examined parameters (including total C-peptide and insulin secretions).
**Conclusion:** The use of different types of calcineurin inhibitors in Type 1 diabetic pancreas and kidney recipients had no effect on glucose metabolism.
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# PS 114
# Hypertension 1

## 1126

**High prevalence of differences in inter-arm blood pressure measurements in patients with Type 2 diabetes**
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**Background and aims:** Determination of blood pressure (BP) differences in both arms is considered important when suspecting abnormalities in the aortic arch or upper-extremity arteries. Recent studies show that the prevalence of a clinically considered relevant difference of 10 mmHg systolic BP occurs in 15% of BP measurements in the general population and in 18% of patients with hypertension. These patients do not necessarily have abnormal pathology, but these findings can have implications for the diagnoses and evaluation of hypertension. We investigated the prevalence of inter-arm BP differences in patients with type 2 diabetes.
**Materials and methods:** Prospective evaluation study in all patients with type 2 diabetes (223 patients) from 5 family practices in which BP was measured according to protocol, using the OMRON HEM-757 automatic BP device. After resting for 5 minutes BP was measured first two times (randomly left or right) on one arm, with a minimal 15 second interval, and two times the other arm.
**Results:** From 207 (93%) patients we have complete data of both arms. The mean BP was 157(± 24)/87(± 12) mmHg. In 107 (57%) patients, an inter-arm BP difference of 10 mmHg or more was found between the first left and right BP. 87 (42%) patients and 18 (9%) patients had a solitary systolic BP or diastolic BP difference of 10 mmHg or more, respectively. In 12 (6%) patients a combined difference of systolic and diastolic BP was found. When comparing the mean of the two left and two right BP measurements we found in 85 (41%) an inter-arm BP difference of 10 mmHg or more, 64 (31%) only systolic, 13 (6%) only diastolic and 8 (4%) combined. The prevalence of a systolic inter-arm BP difference of 10 mmHg or more in one or two measurements is 48% or 35% respectively.
**Conclusion:** Current guidelines often recommend to measure the BP initially in both arms and continue measuring BP on the arm with the highest BP. Often, 10 mmHg BP difference is set as the clinically relevant threshold. More than half of patients has inter-arm BP differences greater than 10 mmHg, which is much more than in the general population. These results give insight into the prevalence of inter-arm BP differences which is relevant for correct diagnosis and follow-up of hypertension, especially in patients with diabetes.

## 1127

**Hypertension in diabetes: conventional or normalised age adjusted cut off's?**
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**Background and aims:** The importance of hypertension in the pathogenesis of diabetic vascular complications is well known. We evaluated microalbuminuria and adverse coronary heart disease risk profile prevalence using conventional (140 and 160 mmHg) and age adjusted centile (75$^{th}$ and 90$^{th}$) systolic BP cut off's (derived from healthy controls) as definitions for hypertension in a whole population with diabetes.
**Subjects and methods:** Data from the Wolverhampton district diabetes register was utilised. Over the 18-month period between Jan 2002–Jun 2003, 4548 individuals with diabetes attended and were studied. 10-year CHD risk scores were calculated using Framingham equation in the primary prevention group. Statistical analysis performed using SPSS version 11.0.
**Results:** The mean age was 60(14) years, BMI 31(6) Kg/m$^2$, duration 12(9) years and systolic BP 147(23) mmHg. Overall, 78% had type 2 diabetes, 55% were on anti-hypertensive therapy, 27% had prior macrovascular event and 34% exceeded albumin/creatinine ratio of 3.5 mg/mmol. The centile cut off's identified more individuals with microalbuminuria and adverse CHD risk in those aged under 50years with a reversal in pattern in the older age groups. In age bands 18–29, 30–39, 40–49, 50–59, 60–69 and >70 years, 75$^{th}$ centile cut off respectively identified 78 %, 69 %, 83 %, 82 %, 79 % and 74 % of those with microalbuminuria and/or CHD scores >15% compared to the 48 %, 62 %, 76 %, 83 %, 87 % and 89 % using conventional cut off of 140 mmHg.

**Conclusion:** The use of control derived age-adjusted cut off's may provide an alternative approach to define hypertension in diabetes that would be of particular value to those under the age of 50years.
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## 1128

**Determinants of ambulatory blood pressure parameters in obese children**
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**Background and aims:** There is little information on the behaviour of night-time and daytime blood pressure (BP) and its associations in obese children. We investigated the relation between ambulatory pressure monitoring (ABPM) parameters, inflammatory markers, glycaemia, insulin, catecholamines and renin-angiotensin system in obese children.
**Materials and methods:** 24 h ABPM was carried out in 82 obese children, BMI 37.3 ± 5.6 kg/m$^2$, waist 109.8 ± 13.6 cm, age range 7–18. Plasma glucose and insulin during an oral glucose tolerance test, as well as fasting lipids, uric acid, CRP, fibrinogen, interleukin 6, renin, aldosterone and 24 h urinary norepineprhine (N) and epinephrine (E) levels were determined. Hypertension was defined by ABPM levels >95$^{th}$ percentile of sex and height adjusted German normality charts.
**Results:** Daytime diastolic (d) ABP was significantly higher than clinic dBP (80.0 ± 7.3 vs 72.9 ± 8.5 mmHg, p<0.0001) while daytime and clinic systolic (s) ABP were similar. On univariate analysis, daytime sABP and dABP were correlated with waist, N, E and uric acid and night-time sABP and dABP were correlated with waist, BMI and uric acid (r 0.253–0.305, p<0.05 and r 0.247–0.286, p<0.05 for daytime and night-time sABP respectively; r 0.294–0.447, p<0.01 and r 0.288–0.434, p<0.001 for daytime and night-time dABP respectively). On multivariate regression analyses, daytime sABP remained independently associated with N (β 0.306, p<0.05), daytime dABP with waist (β 0.352, p<0.01), and both night-time sABP and dABP with BMI (β 0.473, p<0.0001 and β 0.454, p<0.0001 respectively). HR was correlated with 24 h, daytime and night-time s and dABP. No differences were found between obese children with elevated (24%) and those with normal daytime sABP, whereas children with elevated night-time sABP (13%) were more obese (BMI 40.7 ± 4.9 vs 36.1 ± 5.1 kg/m$^2$, p<0.05) and had higher levels of 2 hBG (124.8 ± 18.9 vs 108.4 ± 22.6 mg/dl, p<0.05) than those with normal night-time sABP. Obese children with elevated daytime dABP (20%) had higher levels of interleukin 6 (3.5 ± 2.1 vs 2.3 ± 1.7, p<0.05) and lower levels of upright renin (0.9 ± 0.7 vs 1.4 ± 0.9, p<0.05) than those with normal daytime dABP. Children with elevated night-time dABP (27%) were more obese (39.8 ± 4.3 vs 35.5 ± 5.2 kg/m$^2$, p<0.05) and had higher levels of 2 hBG (123.5 ± 27.6 vs 106.2 ± 18.8 mg/dl, p<0.05) and uric acid (6.9 ± 1.5 vs 5.6 ± 1.6 mg/dl, p<0.01) than obese children with normal night-time dABP. Lipids, insulin, aldosterone, CRP and fibrinogen levels were similar in hypertensive and normotensive obese subjects.
**Conclusion:** In obese children, different mechanisms seem to be associated with elevation of daytime and night-time ABP. Elevated nocturnal ABP is related to the degree of obesity and hyperglycaemia, while adrenergic hyperactivity, low renin levels or inflammation appear to be involved in daytime ABP elevation.

## 1129

**Blood pressure in diabetes: "what are we measuring?"**
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**Background and aims:** World-wide there are various guidelines with different methods on how to measure blood pressure (BP). The cut off point for BP is usually based on large clinical or epidemiological trials. For example, based on the United Kingdom Prospective Diabetes Study (UKPDS), the guideline diabetes mellitus from the Dutch College of General Practitioners (NHG) aim for a BP below 150/85 mmHg in patients with diabetes mellitus type 2. However, the guidelines used by the GP's describe a different method for measuring BP. This can lead to mistakes in the diagnosis and treatment of hypertension. Our objective is to determine differences in BP when comparing the different BP methods.
**Materials and methods:** Prospective evaluation study in all patients with type 2 diabetes (223 patients) from 5 family practices in whom BP was measured according protocol to obtain the following data: A= first reading, B= mean of first 2 readings (JNC7-report), C= 4 readings and mean of last 3 readings (United Kingdom Prospective Diabetes Study), D= mean of first 2 readings with a maximum of 5 mm Hg difference (Dutch Institute of Healthcare).
**Results:** Differences in systolic BP were found between A and B (mean difference 1.6 mmHg, p < 0.001), between B and C (MD 5.7 mmHg, p < 0.001), between B and D (MD 6.2 mmHg, p < 0.001), between A and C (MD 7.3 mm Hg), and between A and D (MD 7.9 mmHg, p<0.001). Also for the diastolic BP differences were found (p<0.001), except between A and B, and between C and D. Of the patients with normal blood pressure according to the UKPDS method (=C), 29.5% would have had hypertension if the guidelines according to the JNC7 report (=B) would had been used.
**Conclusion:** Different methods to assess blood pressure in the same patient lead to significantly different blood pressure readings. These differences are clinically relevant and show an obvious gap between different study methods, guidelines and daily practice. Such differences have clear implications for treatment decisions.

## 1130

**Blood pressure variation and antihypertensive treatment modification in Type 2 diabetes mellitus**
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**Background and aims:** Impaired blood pressure variation i. e. lack of night-time blood pressure fall of minimum 10% ('non-dipping') found in a 24-hour ambulatory blood pressure monitoring (ABPM) is a frequent phenomenon in diabetes as well as hypertension patients, and is considered a risk factor for the development of cardiovascular disease. Circadian blood pressure variation may be affected by blood pressure-lowering agents. Moreover, antihypertensive treatment in type 2 diabetes patients often requires modification so as to comply with ever-changing blood pressure recommended values. The aim of this study was to assess 24-hour blood pressure variation before and after a modification of antihypertensive medication in type 2 diabetes patients.
**Materials and methods:** 59 hypertensive type 2 diabetes patients (24 female, 35 men, mean age 54.6 ± 9.8 years) in antihypertensive treatment was subject to single modification (i. e. either dose increase or decrease or drug introduction or interruption) in effort to to achieve optimal blood pressure control. ABPM was performed twice in each patient: before and 7–10 days after the medication change was made. Mean 24-hour systolic and diastolic blood pressure as well as relative changes of thereof were calculated. Dipping status was assessed on both occasions. Patients who changed their dipping status regardless of its mode ('dipper' into 'non-dipper' or opposite) were labelled 'converters', and those in whom blood pressure variation pattern remained stable as 'non-converters'. Clinical data on the methods and effects of diabetes control were collected.
**Results:** Mean (±SD) systolic and diastolic blood pressure values as well as systolic and diastolic nighttime falls were similar before and after the medication change: 124.3 ± 17.3 vs 122.4 ± 19.1 mmHg; 76.8 ± 10.5 and 77.2 ± 12.1 mmHg; 6.0 ± 7.6 and 5.5 ± 8.7%; 5.8 ± 8.4 and 6.9 ± 8.8%. Mean relative change in systolic blood pressure was 0.4% (range: from −21.4 to 26.9%), diastolic 1.3% (from −28.3 to 42.6%). Non-dipping was found in 45 (76.3%) patients in the first ABPM, and eight out of them (17.8%) converted to dipping in the second ABPM. In addition, three out of 14 'dippers' changed into 'non-dippers'. 'Converters' (n=11; 18.6%) were significantly younger than 'non-converters': 47.5 ± 3.9 vs 56.4 ± 12.2 years; p<0.05) and their systolic blood pressure was significantly lower than in the latter group: 1$^{st}$ ABPM – 115.3 ± 13.1 vs 127.2 ± 17.5 mmHg (p<0.03); 2$^{nd}$ ABPM – 113.7 ± 7.2 vs 127.7 ± 20.3 mmHg (p<0.01), respectively, while diastolic blood pressure was similar in these two groups: 1$^{st}$ ABPM – 77.2 ± 10.3 and 75.4 ± 9.7 mmHg; 2$^{nd}$ ABPM – 77.9 ± 12.9 and 74.9 ± 8.6 mmHg, respectively. No significant differences in diabetes duration, presence of complications, level of blood glucose control as well as antidiabetic and antihypertensive treatment modes were found between 'converters' and 'non-converters'.
**Conclusion:** Impaired blood pressure variation is common in hypertensive type 2 diabetes patients. Dipping status may change in up to 20% type 2 diabetes patients as a result of an antihypertensive medication change. At that time the diagnosis of impaired blood pressure variation should be made with caution, particularly in younger patients with elevated systolic blood pressure.
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## 1131

**Pulse pressure, an important risk factor for atherosclerosis, is significantly increased in Type 2 diabetes: The Sofia Metabolic Syndrome (SMS) study**

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**Background and aims:** Pulse pressure, defined as the difference between systolic and diastolic blood pressure, was recently shown to be an important cardiovascular risk factor. The aim of our study was to examine pulse pressure (PP) in type 2 diabetic patients in comparison to nondiabetics, as well as the relationship of PP to history of myocardial infarction and major atherosclerosis risk factors.
**Material and methods:** A total of 1018 subjects (334 male and 684 female) were included in the Sofia Metabolic Syndrome (SMS) survey, who were consecutive volunteers for a health check examination from the general population, aged above 14 years. All participants filled a questionnaire on family history, medical history and medication, and underwent standardized anthropometrical (body height, weight and waist circumference) and blood pressure measurements. Blood samples were collected and total cholesterol and plasma glucose were examined by routine methods.
**Results:** A total of 8.3% of the study participants had a history of myocardial infarction, 10.3 % had medical history of type 2 diabetes and 41.3% had hypertension. PP was found to be significantly higher ($p=0.033$) in subjects with a history of myocardial infarction ($58.3 \pm 14.6$ mmHg; mean±SD) in comparison to subjects without MI ($54.7 \pm 14.7$ mmHg; mean±SD). In multivariate analysis PP along with age, BMI and waist circumference was found to be a significant risk factor for myocardial infarction. PP was significantly increased ($p<0.001$) in type 2 diabetic patients ($61.3 \pm 16.8$ mmHg; mean±SD) vs. nondiabetic subjects ($53.9 \pm 14.5$ mmHg; mean±SD). PP was significantly correlated with age ($r=0.365$; $p<0.001$), male sex ($r=0.128$; $p<0.001$), body mass index ($r=0.102$; $p=0.006$), waist circumference ($r=0.222$; $p<0.001$) and fasting plasma glucose ($r=0.145$; $p<0.001$). In multiple linear regression analysis age, history of diabetes mellitus and waist circumference were found to be significant independent determinants of PP.
**Conclusion:** Our study demonstrates that pulse pressure is a significant risk factor for myocardial infarction, which is significantly increased in type 2 diabetic patients.
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## 1132

**C-Peptide as a new hypertensive factor, independent of the degree of insulin resistance, in patients with Type 2 diabetes mellitus**

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**Background:** In type 1 diabetics the administration of C-peptide protects against nephropathy. In type 2 diabetics, it has been related with hyperlipidaemia and hypertension although its relationship has not been clarified with respect to Insulin Resistance (IR).
**Aims:**
1. To study the differential characteristics in a type 2 diabetic population without insulin treatment according to fasting C peptide levels.
2. To confirm the existence of a correlation between these levels and Blood Pressure (BP) control and IR.
3. To clarify if C-peptide levels correlate with endothelial dysfunction evaluated by PAI-1 levels.
**Materials and methods:** N=105, 56M, 49F, aged 38 to 82 years, 72 HBP, 51 dyslipemia,16 smokers.
Metabolic markers: HITACHI Autoanalyzer.
Endothelial Dysfunction: PAI-1 Menarini ELISA.
Oxidative stress: oxidized-LDL (oxLDL) Mecordia ELISA.
Plasma insulin (uU / mL) - Immunometric assay. Immulite. DPC.
IR: HOMA score [fasting insulin (uU / mL) × fasting plasma glucose (mmol/L)/22.5].
C-peptide (ng/ml): Immulite 2000 C-peptide.
Hemodynamic markers: ABPM. Spacelabs 90207.
Statistical analysis: t-Student, Chi Square.
**Results:**
1. The population with C-peptide levels >3(ng /ml) had comparable age, HbA1 c and LDLc, but had higher BMI ($p < 0.05$), waist circumference ($p=0.036$), triglycerides($p=0.005$) and IR(p0.039)than population with C-peptide 1–3 ng/ml
2. The group with high C-peptide levels had greater DBP($p=0.001$). C-peptide influences DBP in an independent way to that of IR ($p=0.009$; IR: non significant differences).
3. The group with C-peptide>3 had greater oxidative stress ($p=0.005$) and there is a linear correlation between C-peptide and PAI($r=0.17$;$p=0.033$).
**Conclusion:**
1. In type 2 diabetes patients without insulin treatment, high C-peptide levels correlate with anthropometric parameters, IR and hypertriglyceridemia, but not with the age, LDLc, and HbA1 c.
2. C-peptide influences 24 h DBP, independent of IR association.
3. Patients with high levels of C-peptide had greater endothelial dysfunction and oxidative stress. However, we cannot rule out the implication of BP control and hypertriglyceridemia in this process.

## 1133

**Normal glucose disposal and decreased insulin sensitivity in young normal weight men with non-treated hypertension**

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**Background and aims:** Hypertension is often associated with insulin resistance preferentially in overweight/obese and older subjects. Decreased insulin sensitivity has been observed also in young lean normotensive subjects with family history of hypertension. The aim of our study was to compare insulin and C-peptide responses to i.v. glucose load between young males with early diagnosed mild hypertension or high normal blood pressure (HT) and normotensive controls (NT).
**Materials and methods:** IVGTT (0.3 g glucose/kg body weight during 3 min) was performed in 20 HT aged $22 \pm 1$ (mean ± SEM) years with BMI $23.2 \pm 0.3$ kg/m$^2$ and in 17 gender, age and BMI matched NT aged $24 \pm 1$ years with BMI $22.8 \pm 0.5$ kg/m$^2$. Insulin sensitivity indices (HOMA-IR, QUICKI, NISI) were calculated using plasma glucose, insulin and C-peptide concentrations obtained during IVGTT.
**Results:** Blood pressure was significantly higher in HT subjects compared to NT (135/72 vs. 117/62 mmHg; $p< 0.001$). Insulin resistance was significantly higher (HOMA-IR: $1.9 \pm 0.2$ vs. $1.2 \pm 0.1$, $p= 0.003$) and insulin sensitivity was significantly lower (QUICKI: $0.35 \pm 0.01$ vs. $0.38 \pm 0.01$, $p < 0.001$; NISI: $0.207 \pm 0.002$ vs. $0.219 \pm 0.011$, $p< 0.001$) in HT subjects compared to NT subjects. Insulin (peak: $78.9 \pm 18.2$ vs. $40.4 \pm 7.3$ mU/l, $p= 0.003$, 2-way ANOVA) and C-peptide (peak: $2040 \pm 128$ vs. $1278 \pm 120$ pmol/l, $p < 0.001$, 2-way ANOVA) responses to i.v. glucose load were higher in HT subjects compared to NT subjects.
**Conclusion:** Our results show that normal glucose homeostasis in young lean males with early hypertension was maintained by insulin hypersecretion. Thus, individuals with early-onset hypertension or high normal blood pressure should be considered as subjects at high risk of insulin resistance and its detrimental consequences. Therefore, they represent another target group in which lifestyle managment and periodical control of glucose tolerance and also insulin levels would be recommended.
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## 1134

**FFA levels modulate microvascular function: relevance for obesity-associated insulin resistance, hypertension and microangiopathy**

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**Background and aims:** To test the hypothesis that free fatty acid (FFA) elevation impairs microvascular function and that this contributes to the development of obesity-associated insulin resistance, hypertension and microangiopathy, we examined effects of FFA elevation in lean and of FFA lowering in obese women on skin microvascular function.
**Material and methods:** Sixteen lean and twelve obese women underwent, respectively, Intralipid® plus heparin (or saline) infusion, and overnight acipimox (or placebo) treatment. We measured post-occlusive skin capillary recruitment with capillaroscopy and endothelium-(in)dependent vasodilation by iontophoresis of acetylcholine and sodium nitroprusside in the basal state and during a hyperinsulinemic (40 mU · m$^{-2}$ · min$^{-1}$) clamp.

**Results:** In lean women, FFA elevation impaired capillary recruitment and acetylcholine-mediated vasodilation in the basal state (44.4 ± 18.6 vs. 56.9 ± 18.9%, P<0.05 and 338 ± 131 vs. 557 ± 162%, P<0.01, respectively) and during hyperinsulinemia (54.0 ± 21.3 vs.72.4 ± 25.4%, P<0.01 and 264 ± 186 vs. 685 ± 199%, P<0.01, respectively). In obese women, FFA lowering improved capillary recruitment in the basal state (37.4 ± 9.3 vs.50.9 ± 14.6%, P<0.01) and during hyperinsulinemia (54.8 ± 14.5 vs. 66.8 ± 20.6%, P<0.05). Changes in FFA levels were inversely associated with changes in capillary recruitment and insulin sensitivity in lean women (r=–0.56, P<0.05 and r=–0.61, P<0.05) and in obese women (r=–0.70, P<0.05 and r=–0.74, P<0.01). Subsequent regression analyses showed that, in both lean and obese women, changes in capillary recruitment statistically explained ~29% of the association between changes in FFA levels and insulin sensitivity.
**Conclusions:** These data demonstrate that FFA levels modulate microvascular function and may partially explain FFA-induced insulin resistance in obesity. Therefore, FFA-induced microvascular dysfunction may contribute to the development of obesity-associated insulin resistance, hypertension and microangiopathy.

# PS 115
# Hypertension 2

## 1135

**Determinants of left ventricular dysfunction in hypertensive patients with Type 2 diabetes mellitus**

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**Background and aims:** The aim of the study was to examine the relation between left ventricular systolic dysfunction and major determinants of cardiac mortality and morbidity in hypertensive patients with type 2 diabetes with normal ejection fraction.
**Materials and methods:** We examined 70 hypertensive patients with type 2 diabetes mellitus with ejection fraction > 0.55 and fractional shortening > 0.25 all free of any cardiac symptoms. Thirty-five normal subjects served as controls. Left ventricular longitudinal function was examined by Tissue Doppler and presented as an average of the 16 LV-segments. Peak systolic velocity and strain rate, were obtained in each segment.
**Results:** Patients with diabetes had a significantly lower mean systolic velocities (3.3 ± 1.0 vs. 5.6 ± 1.1 cm/s, $p < 0.001$), as well as systolic strain rate ($-1.1 \pm 0.3\ s^{-1}$ vs. $-1.6 \pm 0.3\ s^{-1}$, $p < 0.001$).
The systolic Doppler measures were correlated to both HbA1 c (r= 0.4, p< 0.01), S-Fructosamine (r= 0.54 p < 0.001), and left ventricular mass (r=0.37 p < 0.01), but not found related to albuminuria, blood pressure (dipping/non-dipping), or anti-diabetic medication. Patients with diastolic dysfunction had significantly higher 24- hour diastolic blood pressure (78 ± 7 vs. 73 ± 7 mmHg, p< 0.01) and the diastolic night and day blood pressure ratio was also significantly correlated to the mitral a-inflow velocity ($\varrho = 0.40$, $p<0.01$).
**Conclusion:** Longitudinal systolic contraction was significantly decreased in hypertensive patients with type 2 diabetes mellitus, with normal ejection fraction, most profound in patients with high HbA1 c, circulating glycocylation end-products and LVH. Diastolic dysfunction was related to non-dipping blood pressure status

## 1136

**Plasma homocysteine levels and arterial hypertension in patients with Type 2 diabetes**

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**Background and aims:** Hyperhomocysteinemia is widely recognized as an emerging risk factor of endothelial dysfunction and vascular damage. Several studies have plasma homocysteine levels linked to blood pressure. There are observations, that homocysteine lowering with folic acid have been followed by decreases in blood pressure.
The aim of this study was to assess the potential associations between homocysteine and blood pressure levels in patients with type 2 diabetes.
**Materials and methods:** Material included 140 patients with type 2 diabetes, among them 105 with arterial hypertension defined as blood pressure values above 140/90 mmHg, or treatment with hypotensive drugs. Serum lipids were determined enzymatically, homocysteine by micro-ELISA, using BioRad reagents, C-reactive protein (CRP) by ultrasensitive immunonephelometric method, vit. B12 and folate level by radioimmunassay. Urinary albumin excretion rate (AER) was determined by immunonephelometry.
**Results:** Mean (SD) age, diabetes duration, body mass index (BMI) and HbA1 c in hypertensive (HT) and normotensive (NT) type 2 diabetic patients were as follows: 61.8 (7.9) vs. 55.6 (8) yrs ($p < 0.05$); 12.4 (7) vs. 10.4 (7) yrs; 30.9 (4.4) vs. 28.3 (4.2) $kg/m^2$ ($p< 0.05$); 7.9(1.4) vs 7.4 (1.3)%. Mean concentration of plasma homocysteine was significantly higher in patients with hypertension than in normotensives (14.6+/–4.6 vs. 11.6+/– 3.2 micromol/ml, p <0.03), while vit. B12 and folate levels did not differ between groups: 432.1(216.4)vs 418.5 (131.3) pg/ml and 3.8(3.4) vs 5.6 (8.0)ng/ml respectively. CRP levels were higher in HT than in NT patients: 2.7(2.9) and 4.7(5.7) mg/l, $p<0.05$. Also AER was higher in HT group than in NT one (35.0 (58.8) vs 14.5 (5.4)microg/ml/min. Mean values of plasma LDL cholesterol were significantly higher in hypertensive patients than

normotensive patients (4.0+/−1.1 vs. 3.3 +/−1.1; p<0.02). Total serum cholesterol, HDL level and triglycerides did not differ between HT and NT groups.
Plasma homocysteine levels in HT group correlated significantly with age ($r = 0.28$, $p < 0.01$)), serum creatinine ($r = 0.47$, $p < 0.001$) and waist to hip ratio ( $r = 0.22$, $p < 0.05$). No significant correlations between homocysteine, blood pressure and folate and vit B 12 levels were observed.
**Conclusion:** The results of the study indicate that hypertensive patients with type 2 diabetes in comparison to patients without hypertension have higher homocysteine, and LDL cholesterol level, two main risk factors of coronary heart disease.

## 1137

### The relationship of platelet 5-HT with hypertension in Type 2 diabetes mellitus

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**Background and aims:** The level of plasma 5-hydroxytryptamine (5-HT) were significantly increased in patients of hypertension, coronary heart disease and cardiovascular incident; the cardiovascular risk factors of type 2 diabetes and hypertension maybe affect the metabolism of platelet 5-HT. We measure the level of platelet 5-HT and plasma 5-hydroxyindoles (5-HIS) in patients of type 2 diabetes and it with hypertension, to study the relationship of the metabolic changes of platelet 5-HT with the cardiovascular risk factors of type 2 diabetes and it with hypertension.
**Materials and methods:** 39 patients with type 2 diabetes group (T2DM), 52 type 2 diabetic patients with hypertension (defined as blood pressure >140/90 mmHg according to WHO) group (T2DM+HBP) and 35 normal controls underwent the determination of 5-HT and 5-HIS concentrations by using the phosphorescence-fluorospectrophotometry.
**Results:** The level of platelet 5-HT in normal controls ($618.36 \pm 194.06$ ng/$10^{-9}$) was higher than that of T2DM ($523.98 \pm 221.37$ ng/$10^{-9}$) and T2DM+HBP ($347.89 \pm 204.84$ ng/$10^{-9}$) (ANOVA P<0.05). Post hoc comparisons of group pairs performed by S-N-K test show that the level in T2DM+HBP was significantly lower than the T2DM (P<0.001) and normal controls (P<0.001) respectively, and the level of platelet 5-HT in T2DM was apparently lower than that of controls (P<0.05). The level of 5-HIS in plasma of normal controls ($183.67 \pm 41.33$ng/ml), T2DM ($161.52 \pm 56.39$ ng / ml) and T2DM+HBP ($169.64 \pm 58.51$ng/ml) were not statistically significantly among the three group (ANOVA, P>0.05).
The change of platelet 5-HT in type 2 diabetic patients were linearity negatively correlated with systolic blood pressure (R=−0.227, P<0.05; Y = 811.11 − 2.697x) and diastolic blood pressure (R=−0.249, P<0.05; Y = 773.40 − 3.908x), and correlated with body weight index (BMI) (P<0.02) and triglycerides (P<0.05); but not significantly correlated with age (years), fasting glucose, $HbA_1C$, creatinine, UREA/Cr, total cholesterol, HDL cholesterol, LDL cholesterol, ApoA and ApoB respectively (p>0.05).
**Conclusion:** The level of platelet 5-HT was decreased in type 2 diabetic patients, especially in diabetes with hypertension. The higher the systolic blood pressure and diastolic blood pressure was, the lower the level of platelet 5-HT in type 2 diabetes patients. The platelet 5-HT correlated with BMI and triglycerides. The level of plasma 5-HIS we measured involved 5-HT and 5-hydroxyindoleaceticacid (5-HIAA), the metabolized products of 5-HT in plasma. And the change of plasma 5-HIS was not apparent, maybe suggest that the activation of platelet and releasing of 5-HT was more important than the action of 5-HT in plasma in the patients of type 2 diabetes and it with hypertension. The relationship was closer between the metabolic changes of platelet 5-HT and the cardiovascular risk factors of type 2 diabetes with hypertension.

## 1138

### Pulse pressure reduction in the AIMEE trial (Addition of Irbesartan 300mg/day to the treatment of Type 2 diabetic hypertensive patients with microalbuminuria, uncontrolled with angiotensin-converting enzyme inhibition)

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**Background and aims:** High pulse pressure (> 60 mmHg) is an important predictor of cardiovascular morbidity and mortality in hypertensive type 2 diabetic patients. Double blockade of the angiotensin-renin system (i. e., combination of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers) has been shown to be a safe and effective therapeutic option with a powerful antihypertensive and antiproteinuric profile, but its effects on pulse pressure have not been studied. The purpose of this work is to characterize these effects in AIMEE, a trial in which Irbesartan was added on top of a previous treatment with angiotensin converting enzyme inhibitors.
**Materials and methods:** 60 patients > 30 years old with type 2 diabetes mellitus, uncontrolled blood pressure (BP > 130/85 mmHg) and persistent microalbuminuria (albumin excretion rate 20–200 µg/min) in spite of full-dose treatment with an angiotensin-converting enzyme inhibitor for at least six months were recruited. Patients with kaliemia > 5 meq/l, plasma creatinine > 176.8 µmol/l (2 mg/dl) or previously treated with angiotensin-receptor blockers were excluded. Blood pressure and heart rate, weight, height and waist perimeter, fasting glycemia, $HbA_{1C}$, sodium, potassium, creatinine, lipid profile and albumin excretion (geometric mean of two or three consecutive overnight collections) were measured by standard means. Irbesartan 300 mg/day was added to the previous treatment of the patients without other changes in the previous antihypertensive treatment. After 2 and 6 months all measurements were repeated; compliance was assessed by pill counting and tolerance by questionnaire.
**Results:** The mean age was $60.1 \pm 15.2$ years; 56.7% were women. All patients completed the first two months of treatment, but 3 (5%) were lost to follow up by the sixth month; 4 (6.7%) had compliance < 80% (but all > 50%). There were no serious side effects, but 2 (3.3%) of the patients had hypotension-related symptoms and were titrated down to Irbesartan 150 mg/day in the 2nd month visit. The decrease in SBP was 19.0 mmHg (17.5 – 20.5, p < 0.001, paired t-test); in DBP was 12.5 mmHg (11.2 – 13.9, p < 0.001, paired t-test). 20% of the patients reached the target blood pressure (p < 0.001, binomial test). Weight, waist perimeter, heart rate, fasting glucose, creatinine, $HbA_{1C}$, sodium and lipid profile did not change significantly. Serum potassium was increased by 0.15 meq/l (p = 0.02, paired t-test) but no patients had clinical hyperkaliemia. Albumin excretion was reduced by 65.7% (61.7 – 69.7, p < 0.001, paired t-test); 36.7% of the patients reached normoalbuminuria. Pulse pressure was reduced from 61.1 ± 6.0 to $54.6 \pm 8.7$ mmHg (p < 0.001, paired t-test); the mean reduction was 6.5 mmHg (5.0 – 8.0). 34 patients (56.7%) had pulse pressure > 60 mmHg at baseline, and 15 (25.0%) by the sixth month (p < 0.001, binomial test).
**Conclusions:** The addition of Irbesartan 300 mg to the treatment of type 2 diabetic patients with uncontrolled hypertension and persistent microalbuminuria in spite of full-dose ACEI was very effective and well tolerated, achieving a substantial reduction in pulse pressure besides a marked improvement in blood pressure and a profound reduction of albumin excretion rate. Most patients with high pulse pressure reached an acceptable one with double blockade of the renin-angiotensin system.

## 1139

### The effect of testosterone on the regulation of the renin angiotensin system in human abdominal subcutaneous adipocytes *in vitro*

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The renin-angiotensin system (RAS) is a key process in the regulation of blood pressure and therefore its' blockade may reduce the vascular complications which arise with polycystic ovary syndrome (PCOS). Adipose tissue expresses all the components of the RAS system that includes the pre-prohormone angiotensinogen (AGT) and its' active metabolite angiotensin II (ANG II), which mediates vasoconstriction and electrolyte balance and as such may contribute to obesity associated hypertension in patients with PCOS. While central adiposity is clearly associated with this risk, limited analysis to date has investigated the direct effects of testosterone on the production of AGT and ANG II. This present study investigated the role of the non-metabolising active testosterone, dihydrotestosterone (DHT), concentration on both the expression of AGT and the secretion of ANG II in subcutaneous (Sc) abdominal fat from women (age 53.2 ± (SEM) 8.3 years; weight 76.3 ± 12.8 kg). Isolated abdominal subcutaneous adipocytes (Sc ad; n=10) were treated with DHT ($10^{-7}$M–$10^{-9}$M) for 48 h. Following treatment, protein was extracted and used to assess AGT by Western blot; while conditioned media were used to assess secretion of ANG II. The effect of DHT ($10^{-7}$M–$10^{-10}$M) on secretion of bradykinin was also assessed. In Sc ad, DHT increased AGT in a concentration dependent manner (control: 1.0 ± (SEM) 0.0; $10^{-7}$M: $2.34 \pm 0.34$***; $10^{-8}$M: $1.85 \pm 0.42$**; $10^{-9}$M: $1.53 \pm 0.22$*; ***p<0.001; **p<0.01; *p<0.05), ANG II levels were also increased in a similar pattern (control: 144 ± (SEM) 4.3; $10^{-7}$M: $437 \pm 15.0$***; $10^{-8}$M: $332 \pm 18.6$***; $10^{-9}$M: $219 \pm 6.9$***). However, bradykinin levels remained unaltered. In summary, these present studies suggest that DHT may increase the local concentrations of AGT and ANG II in isolated adipocytes,

which may contribute to the hypertensive risk in patients with PCOS. Further studies are required to characterise the mechanism by which DHT may alter ANG II levels in this system.

## 1140

**Utility of amlodipine/atorvastatin single-pill therapy in patients with diabetes mellitus: results from the Gemini study**

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**Background and aims:** Patients with diabetes are at high risk of cardiovascular (CV) disease – a risk that is increased further in the presence of hypertension (HTN) and dyslipidemia (DYS). The prevalence of concomitant HTN/DYS is high among patients with diabetes, but attainment of therapeutic goals for these conditions is low. This post-hoc analysis of the Gemini study evaluated the efficacy and safety of amlodipine/atorvastatin single-pill therapy in patients with diabetes and concomitant HTN/DYS.
**Materials and methods:** Gemini was a 14-week, open-label, non-comparative, multicenter trial evaluating the utility, efficacy, and safety of amlodipine/atorvastatin single-pill therapy in patients with concomitant HTN/DYS. Eight dosage strengths of amlodipine/atorvastatin single pill (5/10, 5/20, 5/40, 5/80, 10/10, 10/20, 10/40 and 10/80 mg) were electively titrated to improve patients' blood pressure (BP) and lipid control. This post-hoc analysis included patients with diabetes (controlled to $HbA_{1c} \leq 9.0\%$) who were not at BP or low-density lipoprotein cholesterol (LDL-C) goals at baseline. Patients taking antihypertensive therapies (AHT) and statin lipid-lowering therapy (LLT) prior to screening were not required to undergo a washout. Amlodipine/atorvastatin single pill was added-on to existing non calcium channel blocker (CCB) AHT, substituted in patients who were taking amlodipine or atorvastatin, or switched from LLTs other than atorvastatin or CCBs other than amlodipine. The percentage of patients attaining both BP (JNC VI [<130/85 mm Hg]) and LDL-C (NCEP ATP III [<100 mg/dL]) goals at end point was recorded together with changes in lipid and BP parameters.
**Results:** A total of 227 patients with diabetes received study medication. Of these, 198 patients had previously received AHT (80 of whom were LLT naïve), 132 patients had previously received LLT (14 of whom were AHT naïve), and 15 patients were naïve for both AHT and LLT. Across all patients with diabetes, mean (± SE) systolic/diastolic BP was 146.1 ± 0.8/84.8 ± 0.6 mm Hg and mean LDL-C was 137.4 ± 2.1 mg/dL, at baseline. At end point, 28.4% of patients with diabetes reached their joint BP and LDL-C goals, 43.8% reached BP goal alone, and 55.8% reached LDL-C goal alone. Mean (± SE) change in systolic BP from baseline to end point was −15.5 ± 0.8 mm Hg, and mean percentage change in LDL-C was −27.5 ± 1.3%. Mean dose of study medication at end point was amlodipine 7.73 mg/atorvastatin 30.53 mg. Mean reductions in SBP and LDL-C for the LLT-naïve patients receiving additional AHT were −16.5 ± 1.4 mm Hg and −36.0 ± 2.0%, respectively. At end point, 35.4% of these patients achieved their joint BP and LDL-C goals. Amlodipine/atorvastatin single-pill therapy was well tolerated and most adverse events were mild to moderate in intensity.
**Conclusion:** Simultaneous treatment of HTN and DYS with single-pill amlodipine/atorvastatin therapy offers a novel, effective, and safe method of reducing BP and LDL-C and improving dual goal attainment in patients with diabetes. Effective management of concomitant HTN/DYS is particularly important in this patient subgroup due to their high risk for CVD.
*Supported by Pfizer Inc*

## 1141

**Management of hypertensive patients with and without diabetes in rural Victoria**

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**Background and aims:** Intensive hypertension management in diabetes is associated with reduced complications and blood pressure (BP) targets are below those of people without diabetes. This study compared the hypertension management among rural hypertensive Victorians with and without diabetes.
**Materials and methods:** A 12 month retrospective audit of hypertension management was undertaken in 2000/2001 using a standard template in 3 (of 23) randomly selected general practices in rural Victoria, Australia. Highest blood pressure every quarter (if recorded) was noted. Overall, 3,134 patients were identified with recorded hypertension, of whom 395 (12.6%) had diabetes.
**Results:** Patients with diabetes were older (67 ± 12 v 64 ± 15 <.001). Diabetic patients were more likely to have at least quarterly BP measurements (19.5% vs 12.0 %, p<.001) and a similar proportion had no BP measurements (6.1% vs 4.8 %, p=.267). First diastolic BP, but not systolic BP, was lower in diabetic patients (147 ± 19/81 ± 10 vs 145 ± 18/82 ± 10, p = .074 / .003). Blood pressure generally worsened over the 12 months where quarterly measures were taken. Diabetic patients were more likely to have exercise and weight recorded over the year (8.1% vs 4.2%; 50.0% vs 36.2%, p=.001;<.001). Serum lipids were also marginally more likely to be recorded among diabetic patients (9.1% vs 5.4%, p=.005), but were less likely to be elevated (≥6.0 mmol/l) among those with diabetes (8.3% vs 27.2%). Few patients had microalbuminuria measured. Only 13.4% of those with diabetes and 3.5% of others had either an HbA1 c or glucose recorded. Diabetic patients were more likely to be treated pharmacologically (82.5% vs 63.2%) and with more agents (1.5 ± 1.0 vs 1.0 ± 1.0, p<.001). Only 15.7% of diabetic patients and 7.4% of other patients were treated with 3 or more agents. The most common agent used were ACE inhibitors in either group. Diabetic patients were significantly more likely to be treated with diuretics (32.9% vs 19.0%, p<.001), ACE inhibitors (57.5% vs 34.6%, p<.001), calcium antagonists (31.6% vs 22.5%, p<.001), lipid lowering agents (34.4% vs 18.3%) and aspirin (25.1% vs 16.9%, p<.001). Beta blocker use (15.2% vs 14.0%), Angiotensin II receptor blocker use (7.9% vs 8.8%) and alpha blocker use (1.5% vs 1.8%) were comparable.
**Conclusion:** This study demonstrates that at least in this area, hypertension among diabetic patients remains under-managed, although cardiovascular risk factors are marginally more aggressively treated than among non diabetic patients.

# PS 116

# Treatment of dyslipidemia in diabetes

## 1142

**Multifactorial treatment approach in patients with coronary heart disease and the metabolic syndrome: a GREek Atorvastatin and Cardiovascular Evaluation (GREACE) substudy**

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**Background and aims:** The evaluation of the clinical benefit of statin treatment within a multifactorial treatment approach in patients with coronary heart disease (CHD) and the metabolic syndrome (MetS) with or without diabetes mellitus (DM).
**Materials and methods:** The NCEP-ATP III criteria were used. From 1,600 CHD patients of the GREek-Atorvastatin-and-CHD-Evaluation (GREACE) Study, 682 had the MetS. Patients were divided into two groups: Group A (n=355) included patients on lifestyle advise, drug treatment with statins, and treatment of arterial hypertension and elevated glucose levels and Group B (n=327) patients were on all the above except statins. All patients had lifestyle advice and were followed for a 3-year period. The primary endpoint was "all vascular events" comprising all primary endpoints of the original study.
**Results:** In Group A all patients were on statins and 91% of them (n=324) were at the NCEP LDL-C goal (<100 mg/dL;2.6 mmol/L). In group B 6 (2%) patients reached the LDL-C goal with lifestyle changes. During the study 90 patients (27.5%) of Group B had a primary endpoint vs 43 (12%) of patients in Group A; relative risk reduction=56%,p<0.0001. Event rate curves started deviating from the 6th treatment month, but differences became statistically significant at third treatment year. A statin should be added to other treatments in 6.5 patients for a 3-year period to avoid one cardiovascular event.
**Conclusion:** Lifestyle changes and other treatments are not effective enough in the absence of statin use, at least in CHD patients with the MetS. Aggressive statin treatment reduces cardiovascular events by more than one-half within a 3-year period, in comparison to untreated patients.

## 1143

**Reduction of large VLDL particles by fenofibrate is associated with an improvement of atherogenic lipid profile in Type 2 diabetes**

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**Background and aims:** Elevation of large VLDL particles rich in apo CIII is a dominant feature of diabetic dyslipidemia. Fenofibrate decreases effectively plasma triglycerides (TG) and increases LDL size, but no data are available on its effects on VLDL subspecies. We determined the action of fenofibrate treatment on the subspecies of triglyceride-rich lipoproteins (TRLs) in Type 2 diabetic patients.
**Materials and methods:** 202 subjects with Type 2 diabetes recruited to the FIELD study in Helsinki center were randomly assigned to micronised fenofibrate 200 mg/day or placebo in a double-blind design. The patients were between 50 and 75 years of age, with fasting cholesterol <5.5 mmol/l and TG <5.0 mmol/l before treatment. Mean HbA1C was 7.36 ± 1.4%. The mean follow-up was 24.7 ± 1.4 months in this substudy. VLDL 1 (Sf 60–400), VLDL 2 (Sf 20–60) and IDL (Sf 12–20) were separated by density gradient ultracentrifugation. LDL size was determined with gradient gel electrophoresis. Apo CIII was measured by nephelometry.
**Results:** Fenofibrate reduced by 28% plasma TG levels, the change being mainly due to a marked reduction of VLDL 1 TG (–46%, p<0.001). The concentration of VLDL 2 TG was also reduced by fenofibrate (0.32 ± 0.159 vs. 0.22 ± 0.136 mmol/l, p<0.0001) but IDL TG was similar in fenofibrate and placebo groups. The fenofibrate treatment reduced the mass concentrations of VLDL 1, VLDL 2 and IDL particles, as reflected by significant changes in the concentrations of free cholesterol, cholesterol ester, phospholipids and proteins, the change of VLDL 1 being most pronounced. LDL particle size was larger in the fenofibrate group than placebo treatment group (26.19 ± 0.82 vs. 25.54 ± 1.055 nm, p<0.0001). In the fenofibrate group plasma apo CIII was markedly lower than in placebo group (2.93 ± 1.298 vs. 4.18 ± 2.028 mg/dl, p<0.0001), due to changes of apo CIII both in VLDL 1 (0.56 ± 0.409 vs. 1.04 ± 0.686 mg/dl, p<0.0001) and in VLDL 2 (0.36 ± 0.238 vs. 0.55 ± 0.310 mg/dl, p<0.0001).
**Conclusion:** In Type 2 diabetic patients fenofibrate treatment resulted in a marked reduction of large VLDL particles that is considered to be the culprit of atherogenic lipid profile. Quantitative and qualitative changes of large VLDL particles are associated with beneficial changes of LDL subspecies, improving the atherogenic lipid profile in Type 2 diabetes.

## 1144

**Efficacy and safety of coadministered ezetimibe and fenofibrate in patients with mixed hyperlipidemia**

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**Background and aims:** Mixed hyperlipidemia is a metabolic disorder characterized by elevated levels of low-density lipoprotein cholesterol (LDLC), non-high density lipoprotein cholesterol (non-HDLC), and triglycerides (TG), and reduced levels of HDLC. The cholesterol absorption inhibitor, ezetimibe (EZE) lowers LDLC by approximately 15–25%, while fenofibrate (FENO) is effective in reducing TG and increasing HDLC. Efficacy and safety of coadministered EZE + FENO were examined in patients with mixed hyperlipidemia.
**Materials and methods:** This was a multicenter, randomized, double-blind, placebo-controlled, parallel arm trial. Eligible patients were 18–75 years of age, with mixed hyperlipidemia after a 6–8 week washout (LDLC 3.4–5.7 mmol/L; TG 2.7–5.7 mmol/L), and no history of coronary heart disease (CHD), CHD-equivalent disease (except for type 2 diabetes), or CHD risk >20% as defined by NCEP ATP III criteria. Patients with type 2 diabetes were limited to those with LDLC 2.6 – 4.7 mmol/L after washout. Patients were randomized in a 3:3:3:1 ratio to one of 4 treatment arms for 12 weeks: EZE 10 mg plus FENO 160 mg; FENO 160 mg; EZE 10 mg; or placebo. The primary endpoint compared the LDLC lowering efficacy of EZE+FENO vs. FENO monotherapy.
**Results:** LDLC, non-HDLC, and apolipoprotein B (apo B) were significantly (p<0.001) reduced in the EZE+FENO group compared with the FENO or EZE monotherapy group (Table). HDLC was significantly increased with EZE+FENO and FENO treatments (p<0.001). TG levels were significantly reduced by both EZE+FENO and FENO. All three active treatments were well-tolerated.
**Conclusion:** Coadministration of ezetimibe and fenofibrate provides a complementary efficacy therapy that improves the atherogenic lipid profile of patients with mixed hyperlipidemia. Treatment with EZE+FENO was also well-tolerated with a safety profile comparable to FENO monotherapy.

Percent Change in Lipid Profiles

| Parameter | EZE + FENO N = 183 | FENO N = 188 | EZE N = 185 | Placebo N = 63 |
|---|---|---|---|---|
| Baseline LDLC (mmol/L) | 4.2 ± 0.7 | 4.2 ± 0.7 | 4.1 ± 0.7 | 4.2 ± 0.7 |
| % Change | -20.4 | -5.5[a] | -13.4[a] | 0.2[a] |
| Baseline HDLC (mmol/L) | 1.1 ± 0.2 | 1.1 ± 0.3 | 1.1 ± 0.2 | 1.1 ± 0.2 |
| % Change | 19.0 | 18.8 | 3.9[a] | 3.2[a] |
| Baseline TG# (mmol/L) | 3.1 ± 1.1 | 3.2 ± 1.1 | 3.1 ± 1.2 | 3.0 ± 0.9 |
| % Change | -44.0 | -43.2[b] | -11.1[a] | -9.2[a] |
| Baseline Non-HDLC (mmol/L) | 5.7 ± 0.8 | 5.8 ± 0.8 | 5.6 ± 0.8 | 5.6 ± 0.7 |
| % Change | -30.4 | -16.2[a] | -14.7[a] | -0.2[a] |
| Baseline apo B (g/L) | 1.7 ± 0.2 | 1.7 ± 0.3 | 1.7 ± 0.3 | 1.7 ± 0.2 |
| % Change | -26.1 | -15.2[a] | -11.3[a] | -1.2[a] |

Notes: Baseline data are expressed as mean ± standard deviation (SD), except TG levels are median ± SD#. Percent change data are expressed as LS mean, except TG data are median#. [a]p < 0.001 for EZE+FENO vs. specific treatment group. [b]p = 0.021 for EZE+FENO vs. FENO monotherapy.
*This study was sponsored by Merck-Schering Plough Pharmaceuticals, North Wales, PA.*

## 1145

**Low-density lipoprotein cholesterol goal attainment among patients with diabetes mellitus treated with ezetimibe plus simvastatin coadministration versus simvastatin alone**

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**Background and aims:** Many patients with diabetes mellitus (DM) fail to reach aggressive low-density lipoprotein cholesterol (LDL-C) targets. We assessed whether ezetimibe plus simvastatin (EZE/SIM) would be more effective than SIM alone in helping DM patients achieve an LDL-C goal of <2.6 mmol/L.
**Materials and methods:** This was a post-hoc subgroup analysis of data from a randomized study in which patients with LDL-C ≥ 3.4 mmol/L and meeting NCEP ATP III criteria for CHD/CHD risk equivalent, were randomized to 1 of 4 daily treatments for 23 weeks: SIM 20 mg (n=253); EZE/SIM 10/10 mg/mg (n=251); EZE/SIM 10/20 mg/mg (n=109); EZE/SIM 10/40 mg/mg (n=97). Patients not at LDL-C goal had their SIM dose doubled at weeks 6, 12 and/or 18, up to 80 mg. The subgroup analysis assessed LDL-C goal attainment among patients with (n=342) and without DM (n=368); the primary efficacy objective was % of patients at goal (<2.6 mmol/L) after 5 weeks.
**Results:** In both subgroups (DM and non-DM), more patients treated with EZE/SIM (all doses) than SIM 20 mg, achieved goal after 5 weeks (p<0.001; table), and at 23 weeks (p<0.001). Because DM patients had lower baseline LDL-C than did non-DM patients (4.2 vs 4.6 mmol/L), more DM patients in all treatment groups reached goal; however, after adjusting for baseline differences, goal attainment rates among DM and non-DM patients were not statistically different (p>0.20). EZE plus any SIM dose also produced significantly greater % reductions in LDL-C than did SIM 20 mg (table).
**Conclusion:** Through dual inhibition of cholesterol absorption and synthesis, EZE/SIM offers an effective treatment option for patients with diabetes mellitus.

| | SIM 20 mg | EZE/SIM 10/10 mg/mg | EZE/SIM 10/20 mg/mg | EZE/SIM 10/40 mg/mg |
|---|---|---|---|---|
| **DM Subgroup** | **n=113** | **n=128** | **n=60** | **n=41** |
| Baseline LDL-C (mmol/L) | 4.3 | 4.1 | 4.2 | 4.1 |
| % to LDL-C goal | 53.6 | 83.7* | 86.4* | 92.7* |
| Mean (SE) % LDL-C change | −38.7 (1.3) | −49.5 (1.3)* | −52.6 (1.6)* | −59.2 (1.9)* |
| **Non-DM Subgroup** | **n=140** | **n=123** | **n=49** | **n=56** |
| Baseline LDL-C (mmol/L) | 4.7 | 4.5 | 4.5 | 4.6 |
| % to LDL-C goal | 39.0 | 65.5* | 79.6* | 83.6* |
| Mean (SE) % LDL-C change | −35.2 (1.7) | −42.2 (1.7)+ | −51.4 (2.4)* | −55.1 (2.5)* |

*p<0.001; +p=0.003 compared to SIM 20 mg

*Supported by: Merck/Schering-Plough Pharmaceuticals*

## 1146

**Effects of rosuvastatin and atorvastatin on plasma lipids in Type 2 diabetes patients in the MERCURY I study**

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**Background and Aims:** Statins are first-line lipid-modifying drug therapy in diabetic patients, in whom the primary treatment goal is LDL-C reduction. The MERCURY I study compared the efficacy of open-label rosuvastatin 10 mg (R10), atorvastatin 10 mg (A10), atorvastatin 20 mg (A20), simvastatin 20 mg, and pravastatin 40 mg in achieving LDL-C goals and modifying lipid measures in hypercholesterolemic patients with CHD, atherosclerosis, or type 2 diabetes. After a 6-week dietary lead-in period, 3140 adults were randomized to one of the five treatments for 8 weeks; according to initial randomization, patients either remained on these treatments or switched to rosuvastatin 10 or 20 mg for an additional 8 weeks. The current retrospective subgroup analysis is restricted to the effects of R10, A10, and A20 on plasma lipids in 830 diabetic patients during the first 8-week treatment period.
**Materials and Methods:** Of diabetic patients, 140 randomized to R10, 142 to A10, and 265 to A20 were included in the efficacy analysis. Analysis of variance was used to compare % changes in LDL-C, TC, non-HDL-C, TG, and HDL-C at 8 weeks.
**Results:** Both statins were effective in improving the lipid profile (see table) with R10 producing greater improvements compared with A10 and similar effects to A20. Improvements included a marked reduction in non-HDL-C, an important measure of atherogenic lipoproteins in the setting of the elevated TG common to diabetic dyslipidemia. All treatments were well tolerated; treatment-emergent adverse events occurred in 25.7% of R10 patients, 32.6% of A10 patients, and 32.4% of A20 patients and led to withdrawal in 2.1%, 2.1%, and 3.3%, respectively. No serious treatment-related events were reported.

| | R10 (n=140) | A10 (n = 142) | A20 (n = 265) |
|---|---|---|---|
| **LDL-C** | | | |
| Baseline (mmol/L) | 4.2 (0.7) | 4.2 (0.7) | 4.2 (0.8) |
| % change | −47.2 (1.4) | −37.0 (1.4)* | −44.1 (1.0) |
| **TC** | | | |
| Baseline (mmol/L) | 6.4 (0.8) | 6.3 (0.8) | 6.4 (0.9) |
| % change | −32.9 (1.0) | −25.4 (1.0)* | −31.3 (0.8) |
| **Non-HDL-C** | | | |
| Baseline (mmol/L) | 5.1 (0.9) | 5.1 (0.8) | 5.1 (0.9) |
| % change | −42.3 (1.3) | −32.9 (1.3)* | −40.2 (1.0) |
| **TG** | | | |
| Baseline (mmol/L) | 2.0 (0.8) | 2.0 (0.7) | 1.9 (0.7) |
| % change | −17.3 (2.7) | −11.7 (2.6) | −18.1 (2.0) |
| **HDL-C** | | | |
| Baseline (mmol/L) | 1.2 (0.3) | 1.2 (0.3) | 1.2 (0.3) |
| % change | +6.0 (1.4) | +4.6 (1.4) | +5.6 (1.0) |

Baseline data are mean (SD). Change is least-squares mean % change (SE) from baseline in the intention-to-treat population with last observation carried forward. *P<0.0001 vs. R10 (significance defined as <0.0125 to adjust for multiple comparisons).

**Conclusions:** Rosuvastatin and atorvastatin are effective in modifying the atherogenic lipid profile in hypercholesterolemic patients with type 2 diabetes. Rosuvastatin 10 mg produced greater improvements in the lipid profile compared with atorvastatin 10 mg and similar effects to atorvastatin 20 mg.
*Supported by: AstraZeneca, Alderley Park, Cheshire, United Kingdom.*

## 1147

**A COMparative study with rosuvastatin in subjects with METabolic Syndrome: results of the COMETS study**

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**Background and aims:** The metabolic syndrome increases the risk of coronary heart disease (CHD). COMETS (4522IL/0069) was a double-blind, double-dummy, randomised, multinational, 3-arm, parallel-group study comparing the efficacy of rosuvastatin (RSV) with that of atorvastatin (ATV) and placebo in patients with the metabolic syndrome. The primary objective was to compare percentage changes in LDL cholesterol (LDL-C) in the RSV and ATV groups after 6 weeks' treatment. Secondary objectives included effects on other lipids and high-sensitivity C-reactive protein (hsCRP).
**Materials and methods:** Following a 4-week dietary lead-in period, statin-naïve patients who met the NCEP ATP III definition of the metabolic syndrome, and who had LDL-C ≥3.36 mmol/l (130 mg/dl), additional multiple risk factors conferring a 10-year CHD risk >10% but no evidence of CHD or other atherosclerotic disease or overt diabetes, were randomised (2:2:1) to receive RSV 10 mg, ATV 10 mg or placebo for 6 weeks. Subsequently, the RSV 10 mg and placebo groups received RSV 20 mg and the ATV 10 mg group received ATV 20 mg for a further 6 weeks. Changes in lipids and

hsCRP were assessed at 6 and 12 weeks. Percentage changes from baseline were compared between treatment groups (intention-to-treat population, total=397) using analysis of variance (lipids) and Kruskal-Wallis test (hsCRP). Safety was monitored throughout the study.
**Results:** Mean baseline lipid levels and median baseline hsCRP levels were similar between groups; for LDL-C, HDL cholesterol (HDL-C) and triglycerides (TG), these were 4.4 mmol/l (169 mg/dl), 1.2 mmol/l (45 mg/dl) and 2.3 mmol/l (205 mg/dl), respectively. At 6 weeks, LDL-C was reduced significantly more in RSV 10 mg-treated patients (–41.7%) than in ATV 10 mg-treated patients (–35.7%). Levels of HDL-C (RSV: +9.3%; ATV: +4.8%) and non-HDL-C (RSV: –39.7%; ATV: –34.6%) improved significantly more in the RSV 10 mg group than in the ATV 10 mg group at 6 weeks. At 12 weeks, changes in LDL-C, HDL-C and non-HDL-C were significantly greater in the combined RSV group (RSV 10/20 mg and placebo/RSV 20 mg) compared with the ATV 10/20 mg group. The decreases in TG levels and hsCRP were not significantly different in the RSV group compared with the ATV group. All treatments were well tolerated.
**Conclusion:** RSV had a significantly greater effect than ATV in lowering LDL-C and improving HDL-C and non-HDL-C levels. Beneficial effects on TG and hsCRP were comparable between treatments.

**Least-squares mean change from baseline (%)[a]**

| | 6 weeks | | | 12 weeks | |
|---|---|---|---|---|---|
| | RSV 10 mg (n=164) | ATV 10 mg (n=155) | Placebo (n=78) | RSV 10/20 mg and placebo/RSV 20 mg (n=242) | ATV 10/20 mg (n=155) |
| LDL-C | –41.7 | –35.7*** | –4.1*** | –48.7 | –42.7*** |
| HDL-C | +9.3 | +4.8** | +2.2*** | +10.5 | +5.7** |
| TG | –18.6 | –20.4 | –5.1*** | –23.7 | –23.9 |
| Non-HDL-C | –39.7 | –34.6*** | –4.2*** | –46.6 | –40.8*** |
| hsCRP[b] | –16.6 | –16.7 | –4.2 | –29.0 | –22.6 |

**p<0.01, ***p<0.001 versus RSV at same time point; n=intention-to-treat population; [a]Last observation carried forward; [b]median % change from baseline

*Supported by: AstraZeneca*

## 1148

### Pioglitazone versus gliclazide addition in secondary failure to metformin alone in Type 2 diabetes: beneficial effects of pioglitazone on lipoproteins and inflammation

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**Background and aims:** Type 2 diabetes is associated with accelerated vascular disease, which may be potentiated by dyslipidaemia and inflammation. Secondary failure of glycaemic control with metformin alone can be managed by addition of a thiazolidinedione or sulphonylurea. Response of plasma lipids and inflammation may differ between treatments, in spite of similarly favourable effects on glycaemic control. We determined if addition of pioglitazone vs. gliclazide to metformin in subjects with type 2 diabetes and secondary failure to metformin alone was associated with a more favourable improvement in lipoproteins and inflammatory markers.
**Materials and methods:** A multicentre, double-blind, randomized, parallel group comparison trial of 54 patients with type 2 diabetes and secondary failure to metformin alone was conducted. Subjects (HbA1 c>7.5%) not on lipid-lowering agents were randomized to pioglitazone (15–45 mg, n=26) or gliclazide, (80–320 mg, n=28) for 2-years treatment with a 12-week dose titration period. Lipids were measured by enzymatic assays and LDL-C calculated by the Friedewald equation. ApoA1, ApoB and C-reactive protein (CRP) were measured by nephelometry, and soluble Vascular Cell Adhesion Molecule-1 (sVCAM-1) was measured by ELISA. Results are mean (±SEM) or geometric mean (95% CI). Differences between baseline and end of treatment values were tested by *t* test and between-group differences in change over the treatment period were tested by repeated measures ANOVA. Statistical significance was taken at p<0.05.
**Results:** Baseline concentrations of lipids, apolipoproteins, CRP and sVCAM-1 did not differ between groups. At study endpoint, pioglitazone vs. gliclazide addition was associated with significantly lower triglycerides (TGs), 1.5 (1.3 to 1.8) vs. 1.9 (1.6 to 2.3) mmol/L (p=0.050); higher HDL-C, 1.01 (0.06) vs. 0.82 (0.05) mmol/L (p=0.024); and lower sVCAM-1, 517 (33) vs. 638 (32) ng/mL (p=0.011). The change over time between treatment groups was significant for TGs (p=0.010), HDL-C (p=0.003) and CRP (p=0.026); all trends were more favourable in the pioglitazone group. Total cholesterol, LDL-C, ApoA1 and ApoB did not differ significantly between groups at end of treatment, nor did their rate of change over time differ.
**Conclusions:** In type 2 diabetes with secondary failure on metformin alone, addition of pioglitazone rather than gliclazide results in greater improvements of TG, HDL-C and CRP. Addition of pioglitazone rather than gliclazide to metformin results in a more favourable vascular disease risk factor profile.

## 1149

### Effect of pioglitazone versus gliclazide on serum lipids over two years in patients with Type 2 diabetes

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**Background and aims:** Pioglitazone (PIO) and gliclazide (GLIC) are used as oral antihyperglycemic medications (OAMs) in patients (pts) with type 2 diabetes (T2D). We tested the hypothesis that PIO, by reducing insulin resistance, lowers serum triglycerides (TGs) and raises HDL-C more than GLIC in these pts.
**Materials and methods:** This multicenter, randomized, double-blind, parallel group study is a 2nd year extension of the original study that compared the efficacy of PIO (up to 45 mg QD) with GLIC (up to 160 mg BD) for one year in pts with T2D. In the original study, major inclusion criteria were pts (ages 35–75 years) who had not taken OAM and had an A1C of 7.5–11.0%. In the Extension study at selected sites, inclusion criteria were pts who completed the original study and had an A1C ≤9%. Pioglitazone group had 270 pts (mean age 56 years; BMI=32; duration of DM=2.7 years; A1C=8.6%) and GLIC group had 297 (mean age 56 years; BMI=31; duration of DM=2.9 years; A1C=8.7%). During the 2nd year, pts were discontinued if their A1C ≥ 8%. Fasting serum lipids (TC, HDL-C, LDL-C and TG), glucose, insulin and blood A1C were done at regular intervals throughout the study. Atherogenic index of plasma (AIP) was calculated as log TG/HDL-C.
**Results:** We used analysis of variance with repeated measures to compare the time course of changes in A1C, HOMA-S and serum lipids. Initially, GLIC lowered A1C faster than PIO. At 32 weeks, the A1C curves crossed after which PIO lowered A1C more than GLIC. Pioglitazone improved HOMA-S more than GLIC (p<0.0001). Pioglitazone lowered TG (p<0.045), raised HDL-C (p<0.0001) and reduced AIP (p<0.0001) more than GLIC in a sustained manner over two years. By week 104, LDL-C dropped significantly below baseline in both groups (p<0.0001) with PIO having a higher LDL-C than GLIC by 0.23 mmol/L. HDL-C increased to week 104 in both (p<0.0001) with PIO having a higher HDL-C than GLIC by 0.15 mmol/L.
**Conclusions:** Although PIO and GLIC improve glycemic control in pts with T2D, they differed in their effects on insulin sensitivity and dyslipidemia over two years.

## 1150

### Effect of rosiglitazone on lipid metabolism in Type 2 diabetes mellitus patients who were predominant type B LDL and on statins

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**Background and aims:** In a previous study, rosiglitazone (RSG) increased LDL buoyancy (LDL Rf), large buoyant (lb) LDL and HDL2, and decreased small dense (sd) LDL in type 2 diabetes mellitus (T2DM) patients. Addition of atorvastatin decreased cholesterol in all non-HDL lipoprotein species, but did not modify RSG's induction of lb-LDL and HDL2.
**Materials and methods:** This study determined the effect of RSG on lipid profiles of well-controlled T2DM subjects (n = 72) on a stable dose of a statin for ≥ 2 months, with predominantly sd-LDL (LDL Rf < 0.263), $HbA_{1c}$ 6.36 ± 0.10, mean age 60.4 ± 8.2 years, BMI 31.9 ± 5.4 kg/m$^2$, and male : female ratio 43 : 29. Subjects were randomized to placebo (n = 14), RSG 4 mg/day (n = 29) or RSG 8 mg/day (n = 29) for 12 weeks. Lipids were assessed by standard profile and density gradient ultra centrifugation (DGUC).
**Results:** At study end, RSG significantly increased LDL Rf from baseline in both groups, as well as cholesterol in the HDL subfractions ($P < 0.05$) and lb-LDL subfractions ($P < 0.05$).

LDL-c in sd-LDL subfractions decreased non-significantly in the RSG groups, while triglycerides did not significantly change in either RSG group. Fasting plasma glucose (FPG) and insulin significantly decreased from baseline in the RSG groups.

Mean changes/percent changes* from baseline at week 12 (95% CI)

| Parameters | Placebo | RSG 4 mg/day | RSG 8 mg/day |
|---|---|---|---|
| LDL Rf | -0.0003 (-0.0112, 0.0107) | 0.0110 (0.0033, 0.0187) | 0.0140 (0.0069, 0.0212) |
| *$LDL_{-c}$ | -0.1 (-11.6, 13.0) | 7.1 (-1.8, 16.8) | 10.2 (1.7, 19.4) |
| $LDL_{-c}$/LDLApo-B | -0.002 (-0.053, 0.049) | 0.037 (0.002, 0.073) | 0.049 (0.015, 0.083) |
| FPG (mmol/l) | -0.55 (-1.17, 0.06) | -0.92 (-1.35, -0.49) | -1.41 (-1.84, -1.02) |
| *Insulin | -16.2 (-32.3, 3.8) | -25.4 (-73.6, -32.8) | -25.9 (-35.8, -14.4) |

* Geometric mean in percent change

**Conclusions:** RSG significantly improved lipid profiles by increasing LDL Rf, changing sd- to lb-LDL particles, and increasing HDL-c subfractions in DGUC in T2DM patients already treated with statins.

## 1151

**Favorable long-term effect of rosiglitazone on plasma lipid concentrations in patients with Type 2 diabetes**

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**Background and aims :** Although rosiglitazone, an insulin sensitizer, is known to have beneficial effects on high density lipoprotein (HDL) and low density lipoprotein (LDL) particle size, it has adverse effects on the increment of total cholesterol (TC) and LDL-C in many studies. Such controversial effects of rosiglitaozne on the plasma lipid profiles seem to be attributed to the fact that most studies with rosiglitazone are limited to a short period of follow up. The aim of this study is to evaluate the long term effects of rosiglitazone on the plasma lipid levels and its corresponding factors.
**Materials and methods:** We prospectively evaluated fasting plasma glucose, HbA1 c, TC, LDL-C, triglyceride (TG) and HDL-C at baseline and every three months after rosiglitazone usage (4 mg/d) in 129 (men 92, women 37, age 53 ± 10 yr, BMI 25 ± 3 $kg/m^2$) type 2 diabetic patients. Patients who were treated with any antihyperlipidemic drug during the study period were excluded.
**Results:** Lipid profiles, plasma fasting glucose and HbA1 c are shown in table.

Lipid profiles, plasma fasting glucose and HbA1 c

| | baseline | 6 m | 12 m | 15 m | 18 m |
|---|---|---|---|---|---|
| TC (mmol/L) | 4.63 ± 0.74 | 5.04 ± 0.8 * | 4.93 ± 0.75 *† | 4.71 ± 1.72 † | 4.86 ± 0.97 |
| HDL-C (mmol/L) | 1.11 ± 0.27 | 1.17 ± 0.28 * | 1.24 ± 0.39 *† | 1.28 ± 0.34 | 1.38 ± 0.43 |
| TG (mmol/L) | 1.82 ± 0.79 | 1.86 ± 0.96 | 1.82 ± 0.94 † | 1.87 ± 1.08 | 1.53 ± 0.87 |
| LDL-C (mmol/L) | 2.69 ± 0.67 | 2.96 ± 0.68 * | 2.89 ± 0.71 * | 2.58 ± 1.62 † | 2.78 ± 0.73 |
| FBG (mmol/L) | 8.2 ± 2.3 | 7.1 ± 1.8 * | 6.9 ± 1.7 * | 7.1 ± 1.9 *† | 7.5 ± 2.9 |
| HbA1 c (%) | 7.4 ± 1.3 | 7.2 ± 1.1 * | 7.0 ± 1.0 *† | 7.1 ± 1.4 * | 7.3 ± 1.9 |

Values reported as means± SEM. * $p<0.05$ vs baseline; † $p<0.05$ vs 6 month TC and LDL-C were maximally increased at 6 months but at 12 months TC was decreased and at 15 month LDL-C was decreased significantly. HDL-C was increased significantly from 3 months and the increment was maintained. TG was decreased significantly at 12 months. The elevation of LDL-C during first 6 months showed significant correlation with basal TC (r= -0.357), basal TG (r= 0.342), basal LDL-C (r= -0.501), TC at 12 months minus TC at 6 months (r= -0.390) and LDL-C at 12 months minus LDL-C at 6 months (r= -0.431) ($p< 0.05$).
Multiple regression analysis showed basal LDL-C was an independent corresponding factor for the increment of LDL-C during first 6 months ($p< 0.05$).
The increment of HDL-C showed negative correlation both with basal TC (r= -0.285) and HDL-C (r= -0.362) ($p< 0.05$). Among all parameters, basal HDL-C was the only variable contributing to the elevation of HDL-C during the first 6 months in regression analysis ($p< 0.05$).
**Conclusions:** The hypoglycemic effect of rosiglitazone appears in the relatively early period of treatment (within few months), but favorable effect of rosiglitazone on dyslipidemia seems to appear after somewhat a longer treatment term (probably after 1 year).

# PS 117

# Mechanisms of lipid alterations

## 1152

**Circulating oxidized low-density lipoproteins and antibody to oxidized low-density lipoproteins in Type 2 diabetic subjects: the role of glycoxidation**

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**Background and aims:** Lipoprotein oxidation and glycoxidative stress play an important role in diabetic vascular complication. Antibody against oxidized LDL (oxLDL Ab) occur in human sera, although their biological significance still remains unsolved. To better understand the role of these factors related to atherosclerosis and their relationship, we examined the possible association between LDL physical properties (small and dense LDL are more susceptible to oxidation than larger and more buoyant LDL), the presence of circulating oxidized low density lipoprotein (oxLDL) level and IgG oxLDL Ab titer.
**Materials and methods:** 43 type 2 diabetic subjects, 23 male and 20 female, age 63 ± 9 years (mean±SD), BMI 28 ± 6 Kg/m$^2$, have been studied. The possible role played by glycation and glycoxidation in sensitizing LDL to oxidation was investigated by measuring HbA1 c, and carboxymethyllysine (CML), a major advanced glycation endproduct (AGE). In addition, a marker of NO-dependent damage in vivo, nitrotyrosine, was evaluated. The presence of nitrotyrosine in the plasma proteins is considered indirect evidence of peroxynitrite production. Analyses were conducted using the SPSS statistical analysis package.
**Results:** Our results showed that oxLDL (=42.3 ± 15.0 U/L; mean±SD) did not correlate with LDL cholesterol (=117 ± 48 mg/dl; mean±SD), neither with oxLDL Ab titer (=251.5 ± 159.0 mU/ml; mean±SD) or CML (=1796 ± 397; mean±SD), but we found an association ($p<0.02$) between oxLDL and HbA1 c (=7.0 ± 1.5 %; mean±SD). CML was significantly correlated both with HbA1 c ($p<0.05$) and nitrotyrosine (=4.4 ± 1.8 nM; mean±SD) plasma values ($p<0.006$), but not with plasma fasting glucose (=146 ± 57 mg/dl; mean±SD). Nitrotyrosine was not associated with the other examined parameters, including HbA1 c. In our subjects, IgG oxLDL Ab were not correlated to LDL-Rf (=0.34 ± 0.06; mean±SD), an index of LDL size and density, or to LDL concentration. No differences were seen between male and female.
**Conclusion:** These data indicate that the presence of CML and nitrotyrosine in the plasma of type 2 diabetic subjects (the last one usually not detectable in healthy subjects), even though it shows a possible involvement of glycoxidation and peroxynitrite in the development of diabetic complications, seems not influence LDL oxidation. HbA1 c appears the only parameter which influences LDL oxidation. The production of IgG oxLDL Ab is not affected by LDL levels or LDL physical properties.

## 1153

**Oxidized LDL and other lipid risk factor as a marker for detecting accelerated atherosclerosis in patients with Type 2 diabetes**

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**Background and aims:** Type 2 diabetes is associated with excessive cardiovascular morbidity and mortality. The oxidative conversion of LDL to oxidized LDL (oxLDL) is now considered to be an essential step in the atherogenic process. The aim of this study was to estimate relations between ox LDL and other lipid risk factors for detecting accelerated atherosclerosis in patients with type 2 diabetes.
**Materials and methods:** Investigation was performed in 42 well controled diabetics type 2 patients (mean age: 53.49 ± 6.29 years; HbA1 c<6.5%; FBG<5.5 mmol/l) before any coronary heart disease (CHD) events (group A) and in 32 hypercholesterolemic subjects (mean age: 52,33 ± 3,92 years) without CHD (group B). Triglycerides, total-C and HDL-C were measured by enzymatic methods. LDL-C was calculated using the Friedewald formula. CRP was measured using Behring Latex hs-CRP assay. Ox-LDL was determined by a commercially available sandwich ELISA (Mercodia AB, Uppsala, Sweden)
**Results:** Levels of circulating oxidized LDL was significantly higher in group A than in group B (112.09 ± 41.05 vs 90.75 ± 24.59IU/l; $p=0.02$). Conventional lipid profile (total C, LDL-C, HDL-C. total-C/HDL ratio and LDL/HDL ratio) were similar between groups A and B. Levels of Tg (2.93 ± 2.13 vs.1.71 ± 0.92 $p=0.01$) and Tg/HDL ratio (3.37 ± 2.6 vs. 1.68 ± 1.27 $p=0.02$) were significantly higher in group A. We analyzed correlations between ox LDL and other lipid. We found that in group A oxLDL significantly correlated with Tg ($r=0.484$; $p=0.03$), total-C/HDL ratio ($r=0.559$; $p=0.04$) and Tg/HDL ratio ($r=0.648$; $p=0.01$). In group A we also found significant correlations between Tg/HDL ratio and CRP ($r=0.460$; $p=0.04$). In group B we found that oxLDL only significantly correlated with CRP ($r=0.758$; $p=0.01$). Distribution of oxLDL and other lipid risk factor (total-C/HDL ratio and LDL/HDL ratio), showed that 72.5% of the subjects with high circulating oxLDL had a high risk for CHD (Quartile IV/4).
**Conclusion:** It is vitally important to identify diabetic type 2 patients with accelerated atherosclerosis, because aggressive therapy with drugs, such as statins, could lower the oxidized LDL levels.

## 1154

**Elevated serum malondialdehyde-modified LDL cholesterol concentration in Japanese metabolic syndrome**

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**Background and aims:** It has been reported that the patients with cardiovascular disease have higher serum malondialdehyde-modified LDL cholesterol (MDA-LDL) level, which is one of the oxidatively-modified LDL cholesterol, and it correlates well with both serum LDL cholesterol level and serum triglyceride level. Also, recent research progress showed that visceral fat accumulation or insulin resistance is on the upstream of atherosclerosis and they are called as "metabolic syndrome". We studied the relation between serum MDA-LDL concentration and such syndrome to reveal the significance of measurement of serum MDA-LDL as risk marker for atherosclerosis.
**Materials and methods:** We studied 88 subjects who visited Saiseikai Central Hospital, Tokyo, for routine medical check up (65 male,23 female). All participants were performed 75g OGTT and were measured waist and hip circumference. Serum MDA-LDL concentration was measured by sandwich ELISA using monoclonal antibody. Fat CT at umbilical level was performed in 42 subjects who consent the examination. According to the Japan Society for the Study of Obesity, we defined the visceral obesity as whose waist circumference is over 85 cm for male, and over 90 cm for female, respectively. We also defined "metabolic syndrome" which has three or more abnormality of five component traits (visceral obesity, blood pressure, glucose tolerance, HDL, and triglyceride). We analyzed the relation between the serum MDA-LDL level and these variables.
**Results:** Mean age of study participants were 57.6 ± 10.4 years. Their glucose tolerance revealed normal in 48, impaired fasting glucose in 6, impaired glucose tolerance in 23, and diabetic pattern in 11 subjects, respectively. Subjects with metabolic syndrome revealed higher serum MDA-LDL concentration compared to subjects without metabolic syndrome (116.8 ± 27.6 v.s. 98.1 ± 34.2, $p<0.05$). When we analyzed each component, subjects with either visceral obesity or hypertriglyceridemia showed significantly higher serum MDA-LDL level, however neither low HDL, hypertension, nor glucose tolerance showed significant influence on serum MDA-LDL concentration. Serum MDA-LDL level revealed significant correlation with waist circumference ($r=0.308$, $p<0.01$) and serum adiponectin concentration ($r= -0.330$, $p<0.01$). Both visceral obesity and hypertriglyceridemia independently showed significant relation with serum MDA-LDL level in multiple linear regression analysis. Among the subjects who took fat CT, serum MDA-LDL level is significantly higher in the subjects whose visceral fat mass is more than 100 cm$^2$ ($p < 0.05$).
**Conclusion:** We revealed that serum MDA-LDL concentration was higher in metabolic syndrome in this study. Especially, both visceral obesity and hypertriglyceridemia is independently related to serum MDA-LDL concentration. These two risk factors are known as risky combination for atherosclerosis and called hypertriglycerideamic waist. Our results may suggest that increased lipoprotein oxidation in metabolic syndrome and the relation between serum MDA-LDL and hypertriglycerideamic waist.

## 1155

**Enhanced angiotensin II-mediated proliferative and atherogenic responses in fibroblasts of patients with familial hypercholesterolemia**

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**Background and aims:** Familial hypercholesterolemia (FH) is characterized by an high morbidity and mortality for atherosclerosis. Beside its traditional vasoconscrictor effect, angiotensin II (AngII) is a powerful stimulator of cell proliferation, and induces the synthesis of several growth factors. Clinical and experimental evidence suggest an important role for AngII in the fibrotic response to tissue injury, and in promoting myocardial hypertrophy via paracrine mechanisms mediated by fibroblasts. The aim of this work was to determine whether AngII plays a role in promoting proliferative and pro-atherogenic responses in FH patients.
**Materials and methods:** Fibroblasts (Fi) from cutaneous skin biopsies performed in five patients with heterozygous FH (FH) and five control subjects (C) were used to study the time- and dose-dependent effect of AngII on cell growth (count by hemocytometer), intracellular calcium fluxes (microfluorimetric technique with the probe Fura2/AM), and on expression/release of matrix components, pro-inflammatory peptides and metalloproteases involved in plaque remodeling and vulnerability (RT-PCR and immunoassays). Results were analyzed by 3-way ANOVA and Bonferroni-Dunn test.
**Results:** AngII stimulated cell replication ($5.1 \pm 0.03$ vs $3.2 \pm 0.04$ cells/$50^4$ cells/well, $p<0.0001$), and dose-dependently induced a larger increase in intracellular calcium content in FHFi than in CFi (mean difference=76 nM, $p<0.0001$). Similarly, TGFβ1 and endothelin-1 (ET-1) expression and release were potentiated (after 24-hour incubation with 1 μM AngII TGFβ1 expression was 129 in CFi and 143 AU in FHFi, and its release was $190 \pm 12$ in CFi and $376 \pm 9$ pg/ml/$10^6$ cells in FHFi; ET-1 expression was 127 in CFi and 139 AU in FHFi, and its release was $93 \pm 5$ in CFi and $192 \pm 7$ pmol / ml / $10^6$ cells in FHFi, $p<0.0001$ for both). MMP-1 (that contributes to to the expansion and rupture of the plaque) and MMP-2 (that modulates extracellular matrix remodeling) were increased in FHFi vs CFi ($0.52 \pm 0.04$ vs $0.36 \pm 0.05$ and $24 \pm 4$ vs $13 \pm 3$ ng/mg protein with 1μM AngII, $p<0.0001$ for both). Irbesartan, a selective AT1 receptor antagonist, was able to reduce these responses by 90% in both CFi and FHFi, indirectly confirming a main role of AT1 receptors in mediating the above-described effects.
**Conclusion:** These results show that several intracellular metabolic pathways mediated by AngII are enhanced in fibroblasts from FH patients, independently of the presence of LDL cholesterol; AngII could however play a particularly relevant role in the the development and progression of the atherosclerotic disease in these patients, either through direct mechanisms or endothelin-1-mediated pathways.

## 1156

**High dose thiamine therapy counters diabetic dyslipidemia**

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**Background and aims:** The streptozotocin-induced (STZ) diabetic rat experimental model of diabetes on insulin maintenance therapy exhibits dyslipidemia characterised by increased non-HDL cholesterol, decreased HDL cholesterol and increased plasma triglycerides. The STZ diabetic rat suffers mild thiamine deficiency that may influence dietary lipid uptake and metabolism. We investigated the effect of high dose therapy with thiamine and the thiamine monophosphate derivative Benfotiamine (Bft) on diabetic dyslipidemia in this experimental model.
**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 hyperglycemia was stabilised by injection s.c. of 2 U of Ultralente insulin every 2 days. Thiamine and Bft 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; 8 – 13 rats in each group. Dyslipidemia was assessed by measurement of plasma total cholesterol, HDL cholesterol and triglycerides. Maintenance of the diabetic state was assessed by the assay of plasma glucose concentration and glycated hemoglobin $HbA_1$. Hepatic transketolase and UDP-N-acetylglucosamine were determined at the end of the study by conventional spectrophotometric and anion exchange HPLC procedures, respectively.
**Results:** Plasma glucose concentration was increased 5-fold and $HbA_1$ 2-fold in STZ diabetic rats, with respect to controls ($P<0.001$). Neither plasma glucose concentration nor $HbA_1$ were decreased by thiamine or Bft therapy. In STZ diabetic rats, plasma non-HDL cholesterol was increased 69%, HDL cholesterol was decreased 27% and triglycerides increased 307%, with respect to normal controls. Thiamine therapy (70 mg/kg) prevented diabetes-induced increases in plasma cholesterol and triglycerides in diabetic rats but did not reverse the diabetes-induced decrease of HDL. This was achieved by prevention of thiamine depletion and decreased transketolase activity in the liver of diabetic rats. There was a concomitant decrease in hepatic UDP-N-acetylglucosamine. A lower dose of thiamine (7 mg/kg) and Bft (7 and 70 mg/kg) were ineffective.
**Conclusion:** High dose thiamine therapy prevented diabetic dyslipidemia in experimental diabetes probably by suppression of hexosamine pathway signalling but other factors may also be involved. Benfotiamine may be less effective than thiamine because of decreased delivery of thiamine to the liver by Benfotiamine.
*We thank the Juvenile Diabetes Research Fund and the Wellcome Trust (U.K.) for support for research.*

# PS 118

# Inflammation and cardiac complications

## 1157

**Increased C-jun N terminal kinase (JNK) activity may link insulin resistance and inflammation in human central obesity**

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**Background and aims:** Macrovascular disease is the major contributor to mortality in type 2 diabetes mellitus (T2DM) patients. Central obesity is known to be a major risk factor for the development of T2DM and macrovascular disease, as it is strongly associated with insulin resistance and chronic sub-clinical inflammation. However, the mechanisms underlying this link remain unclear. Recently JNK, a mitogen activated protein kinase (MAPK), has been implicated in obesity and insulin resistance by interfering with insulin action. Studies in mouse models show that phosphorylated JNK-1 is elevated in obesity, whilst in JNK-1 knockout models mice exhibit decreased adiposity, improved insulin sensitivity and enhanced insulin receptor signalling capacity. Furthermore, JNK is activated by TNF-α and forms a pivotal role in the inflammatory response, which suggests that other pro-inflammatory factors, such as IL-6 and leptin, may recruit JNK. This also indicates a potential pathway through which the interaction of adipose tissue products may precipitate sub-clinical inflammation and insulin resistance and the resulting complications.
**Materials and Methods:** Therefore we examined *ex vivo* human adipose tissue from patients undergoing elective surgery (subcutaneous: n=35; omental: n=15; thigh: n=18; Age 45 ± 3.3SD, BMI 21.9 ± 2.4). Firstly, JNK expression and activity were determined in the various adipose tissue depots. In addition, mRNA expression of potential mediators of JNK such as TNF-α, IL-6 and leptin were determined via real-time PCR in fat depots. Our data showed that JNK is expressed in subcutaneous (Sc) adipose tissue, Sc adipocytes and macrophages (control) with the two main forms of JNK located in *ex vivo* fat, JNK1 and JNK2 (43 kDa and 54 kDa respectively). We examined the effect of fat depot on total and phosphorylated protein expression of JNK by western blot and ELISA.
**Results:** Our findings indicated depot specific alteration of phosphorylated JNK, with increased expression in abdominal subcutaneous (Sc) and omental (Om) depots compared with thigh (Sc: 2.6 ± 0.15 & Om: 3.1 ± 0.14; Vs thigh: 1.1 ± 0.1, $p<0.01$). Phosphorylated JNK-1 was specifically increased 2.6 fold from abdominal tissue compared with thigh ($p<0.01$). The mRNA expression studies also demonstrate that the pro-inflammatory factors are elevated in abdominal fat compared to thigh for TNFα, IL-6 and leptin ($p<0.01$).
**Conclusions:** In summary, JNK-1 activity is increased in central abdominal fat. In addition, TNF-α, IL-6 and leptin are increased in abdominal fat thus suggesting that JNK activity may be related to the activation of these pro-inflammatory cytokines. JNK may therefore link human central obesity with insulin resistance and inflammation and thus may represent an important role in the pathogenesis of T2DM and macrovascular complications.

## 1158

**Association of inflammatory markers with concomitant hypertension and parameters of metabolic control in non-obese patients with newly diagnosed diabetes**

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**Background and aim:** Hypertension very often accompanies diabetes and significantly contributes to cardiovascular complications. Current understanding of the pathogenesis of atherosclerosis is based on the inflammatory theory with interleukin 6 (IL6) and C-reactive protein (CRP) as the main markers. They increase with duration of diabetes and poor metabolic control. Therefore we have undertaken a study to assess the relations between serum levels of inflammatory markers and concomitant hypertension and metabolic control parameters in newly diagnosed non-obese diabetics.
**Materials and methods:** From 216 patients (age 25–65) with newly diagnosed diabetes in year 2003 following groups were excluded to eliminate other factors affecting the inflammatory markers: obese (BMI >31), current smokers, patients with recent infections, suffering from chronic inflammatory or autoimmune disorders (asthma, COPD, rheumatoid arthritis, lupus etc.). Finally 52 patients (24 females) were enrolled, 22 age, sex and BMI matched healthy volunteers composed the control group. A detailed history and medical examination were performed, a blood sample was taken in order to asses the serum levels of total cholesterol and its fractions, triglycerides, hemoglobin A1 c, fasting and post-prandial glucose as well as IL6, soluble form of IL6 receptor (IL6R) (ELISA, R&D Systems) and hs-CRP. Mann-Whitney and Spearman tests were used to assess the differences or correlations between the groups, respectively.
**Results:** There were no major differences between groups in levels of IL6 and CRP, whereas IL6R was significantly higher in diabetics (43.8 ± 12.6 vs 36.4 ± 9.9 $p<0.02$). Among 52 patients with diabetes 26 were already diagnosed with hypertension. Patients without hypertension did not differ significantly from controls in antropometric parameters, IL6, IL6R and CRP serum levels. They, however, had significantly lower BMI (25.84 ± 2.8 vs 28.15 ± 2.2 $p<0.005$ and CRP concentration than the group with diabetes and hypertension. Further, correlations between inflammatory markers and antropometric and metabolic control parameters were assessed. Serum IL6 concentration positively correlated with fasting glucose ($R=0.54$ $p<0.005$) and CRP ($R=0.55$ $p<0.005$). CRP levels were associated with post-prandial glucose ($R=0.59$ $p<0.05$). IL6R correlated with BMI ($R=0.304$ $p<0.01$). None of the markers correlated with hemoglobin A1 c.

Table 1. Levels of inflammatory markers. *p<0.05 vs controls #p<0.05 vs diabetics without HA

| | N | IL6 | IL6R | CRP |
|---|---|---|---|---|
| Controls | 22 | 1.86 ± 2.1 | 36.4 ± 9.2 | 8.87 ± 8.8 |
| Diabetics without hypertension | 26 | 2.05 ± 1.9 | 41.4 ± 10.8 | 9.59 ± 12.9 |
| Diabetics with hypertension | 26 | 2.54 ± 2.2* | 45.4 ± 13.8* 33.64 ± 86.5# | |
| All diabetics | 52 | 2.29 ± 2.1 | 43.43 ± 12.5* | 21.8 ± 63.1 |

**Conclusion:** Hypertension markedly increases levels of inflammatory markers in patients with recently diagnosed diabetes and thus may significantly contribute to the acceleration of atherosclerosis and cardiovascular morbidity. Correlation between IL6 and fasting glucose and between CRP and post-prandial glucose levels may imply an association between concentration of inflammatory markers and insulin resistance in non-obese patients with newly diagnosed diabetes.

## 1159

**CRP, fibrinogen, and white blood cell count in patients with stable coronary artery disease and impaired glucose tolerance**

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**Background:** Inflammatory markers are elevated in patients with diabetes mellitus and in patients with coronary artery disease (CAD). However, data are scarce on inflammatory markers in patients with both CAD and diabetes. As impaired glucose tolerance (IGT) frequently precedes diabetes type 2 (DM2), inflammatory markers in patients with IGT are of particular interest.
**Methods:** We measured CRP, fibrinogen, and white blood cell count (WBC) in 468 patients with clinically stable, angiographically proven CAD. Patients with diabetes type 1 (n = 3) were excluded from the analyses. Oral glucose tolerance tests (oGTT) were performed in patients without established diabetes.
**Results:** DM2 had previously been established in 100 patients. In oGTT another 34 patients proved diabetic, 69 had IGT, and glucose tolerance was normal in 262 patients. CRP, fibrinogen, and WBC were similar in patients with established diabetes and in patients with newly diagnosed diabetes; these patients were thus pooled to a single diabetic group. Between patients with normal glucose tolerance and with IGT, CRP (0.30 ± 0.53 vs. 0.30 ± 0.60 mg/dl; p = 0.822), fibrinogen (377 ± 68 vs. 386 ± 65 mg/dl; p = 0.206), and white blood cell count (6.5 ± 1.8 vs. 6.8 ± 2.2 G/l; p = 0.741) were not significantly different. However, all these inflammatory markers were significantly elevated in patients with DM2 (0.49 ± 0.68 mg / dl, 412 ± 83 mg / dl, 7.5 ± 2.1 G/l; p values were ≤ 0.001 for the comparisons vs. normal glucose tolerance and 0.014, 0.102, and 0.007 for the comparisons vs. IGT).

In leukocyte subtype analyses only neutrophils were significantly higher in diabetic patients than in patients with normal glucose tolerance and IGT (4.5 ± 1.4 vs. 3.7 ± 1.1 and 4.0 ± 1.7 G/l; $p < 0.001$ and $p = 0.015$, respectively).
**Conclusions:** Among patients with clinically stable and angiographically proven CAD, CRP, fibrinogen, and white blood cell count are significantly higher in diabetic than in non-diabetic patients, but patients with IGT do not significantly differ from non-diabetic individuals. Specifically, an increase of neutrophils accounts for the overall increase of white blood cell count in diabetic patients with stable CAD.

## 1160

**Adiponectin: a potential mediator of pro-inflammatory cytokine inhibition in epicardial fat from patients undergoing CABG.**
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**Background and aims:** Chronic sub-clinical inflammation is observed in patients that have undergone coronary artery bypass grafting (CABG) and is associated with central adiposity and increased type 2 diabetes risk. Particularly omental adiposity has shown close association with insulin resistance and the production of pathogenic pro-inflammatory markers such as TNFα and IL-6. In contrast, adiponectin represents an adipocytokine associated with increased insulin sensitivity, which also has anti-inflammatory properties. Epicardial fat, which is conserved during starvation, may represent an important source of substrates for myocardial metabolism but may also be important for the regulation of myocardial metabolism through the secretion of paracrine factors.
**Materials and methods:** We studied the mRNA expression of adipocytokines in epicardial fat to investigate the relationship with central abdominal and gluteo-femoral fat tissue. Further to examine the relationship with circulating adipocyokine levels and epicardial mRNA expression. Real time PCR examined mRNA expression of adiponectin, TNFα, IL-6, leptin, resistin, PAI-1 and AGT from CABG patients (Age: 64±(SD)9 yrs; BMI: 27.3 ± 3.3 kg/m$^2$; n=50) compared with adipose tissue from control non-obese subjects (Age: 42 ± 10 yrs; BMI: 26.03 ± 1.8 kg/m$^2$; n=68): comprising of abdominal subcutaneous (Abd Sc), omental, (Om) and thigh tissue. A marker of macrophages was also investigated (CD45). Serum was also taken from a sub-cohort of CABG patients to measure circulating pro-inflammatory cytokines.
**Results and conclusions:** The mRNA expression of adiponectin was found to be significantly raised in epicardial adipose tissue (ΔCt:9.86 ±(SEM)0.23) compared with omental fat (ΔCt:11.83 ± 0.24;p<0.01) from normal control subjects; whilst similar to other Sc sites. There was comparable mRNA expression of AGT, PAI-1 and resistin in epicardial fat compared with omental adipose tissue, while CD45 mRNA expression was markedly increased (p<0.001). In contrast IL-6, leptin, and TNFα were significantly reduced compared with omental adipose tissue (p<0.001). Circulating pro-inflammatory cytokine levels were significantly increased in CABG patients compared to healthy controls (TNFα: Control subjects: 38.6 ± 4.5 pg/mL vs CABG: 64 ± 7.7 pg/mL***; ***p<0.0001; IL-6: Control subjects: 56 ± 7.9 pg/mL vs CABG 104 ± 46 pg/mL**, Resistin: 12.6 ± 3.3 ng/mL vs CABG: 19.8 ± 4.56 ng/mL**; **p<0.001). Epicardial fat expresses a distinct pattern of adipose tissue cytokine genes. Adiponectin mRNA was highly expressed in the epicardial fat depot, compared with omental fat, whilst mRNA expression of pro-inflammatory agents such as IL-6, leptin and TNFα were significantly reduced compared with omental. In contrast, circulating levels of the pro-inflammatory cytokines were increased in CABG patients. The AGT, PAI-1 and resistin mRNA expression in epicardial fat was comparable to omental fat, whilst CD45 was significantly increased. Resistin did not appear to be affected by adiponectin expression in adipose tissue, and its increased expression may relate to infiltration of macrophages. The high mRNA adiponectin levels in epicardial fat is however surprising and suggests that this depot remains insulin sensitive and explains the associated reduction of pro-inflammatory factors such as IL-6, TNFα and leptin.
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## 1161

**Plasma homocysteine levels and chronic complications in patients with Type 2 diabetes**
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**Background and aims:** Hyperhomocysteinemia is recognised as a risk factor of diabetic macroangiopathy, however its associations with microvascular complications are less known. The aim of this study was to assess the relationships between chronic diabetes complications and homocysteine levels in patients with type 2 diabetes.
**Materials and methods:** Material included 140 patients with type 2 diabetes, 71 men and 69 women, recruited from the diabetes outpatient clinic. Macroangiopathy was considered in patients with a history of cardiovascular event, ischemic ecg, with claudication/abolished peripheral pulses, or with carotid disease assessed by Doppler echography. Retinopathy was diagnosed by direct ophtalmoscopy, peripheral neuropathy by questionnaire and clinical examination (abnormal knee/ankle reflexes, monofilament). Microalbuminuria, defined as urinary albumin excretion rate between 20–200 mg/l, was assessed by immunonephelometry. Plasma homocysteine (HC) level was determined by ELISA, using BioRad reagents, serum lipids enzymatically, C-reactive protein (CRP) by ultrasensitive immunonephelometric method and glycated hemoglobin by HPLC. Statistical analysis included receiver operating curve (ROC) analysis and canonical correlation, which allows to examine the associations between two sets of variables.
**Results:** Mean age of patients was 61.0+/–3.1 yrs, mean diabetes duration 12,1+/–6.9 yrs, body mass index (BMI) 30.7+/–4.5 kg/m$^2$ and mean Hba1 c levels 7.9.+/–1.4%. Mean values of serum HC were significantly higher in patients with neuropathy (17.0+/–9.8 micromol/l) and in patients with peripheral macroangiopathy (19.3+/–3.8 micromol/l) then in persons without these complications (13.4+/–4.1 and 13.8+/–4.3 micromol/l respectively). ROC analysis revealed, that for neuropathy the optimal discriminating cut-off point of HC level, as determined by a balance of sensitivity and specificity, was 14,4 micromol/l, with area under ROC curve of 0.78, sensitivity of 75% and specificity of 72%. The optimal cut point of HC concentration for coronary artery diseases was 14.3 micromol/l, with area under ROC curve of 0.72, sensitivity of 56,6% and specificity of 72%.
We assessed the relationship between a set of chronic macro- and microvascular complications and a set of risk factors of these complications such as age, diabetes duration, glycated hemoglobin, BMI, creatinine, homocysteine and CRP. Canonical correlation coefficient was high, indicating significant associations between examined sets of variables, ($R = 0.75$, $p < 0.05$). Among examined variables, the most important role in this association had in a group of chronic complications neuropathy (structure coefficient = 0.907), then retinopathy, nephropathy, coronary artery disease and peripheral macroangiopathy and in a group of risk factors of the complications homocysteine (structure coefficient = 0.789), and then diabetes duration, BMI, serum creatinine, glycated hemoglobin, age and CRP.
**Conclusion:** The results of this study suggest the important role of homocysteine in the development of a set of chronic complications in patients with type 2 diabetes.

## 1162

**Emerging risk factors for coronary heart disease in patients with Type 2 diabetes mellitus**
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**Background and aims:** A number of emerging risk factors for atherosclerosis have recently been proposed and studied. The aim of the study was to investigate such emerging risk factors for coronary heart disease (CHD) in patients with type 2 Diabetes Mellitus (DM).
**Materials and methods:** A total number of 307 patients (181 men, 126 women with a mean age of 62,25 ± 11,84 years) with CHD undergoing coronary angiography were studied during the period of March 2001–December 2003. One hundred eighty four of them (D) had type 2 diabetes and 123 were non-diabetics (nD). Data concerning new or emerging risk factors were measured: Thrombomoduline (TM), Fibrinogen (Fg), von Willebrand factor (vWf), apolipoproteins A,B,E (apo-A, apo-B, apo-E), lipoprotein A (LpA), antithrombine III (ATIII), proteins AI-1, C and S (PAI-1, P-C, P-S), Homocysteine (Hom), C-Reactive Protein (CRP) and Uric Acid (UA) levels

from all 307 individuals. Statistical analysis was performed using one-way Analysis of Variables (AnOVa), regression analysis and non-parametric tests in the SPSS 11.5 programme.
**Results:** In D group mean value of HbA1 c was 7,09 ± 3,41 and mean duration of diabetes was 11,05 ± 6,38 years. Division of subjects according to the number of coronary arteries with evidenced obstruction (C.Art) was of similar percentages for each group (D: 1 C.Art.:31,7%, 2 C.Art.:23,2%, 3 C.Art.:36,6%, nD: 1 C.Art.: 37,4%, 2 C.Art.:26,0%, 3 C.Art.: 28,5%, N/S). Comparison between D and nD group revealed several risk factors of statistical significance: **Fg** (D vs nD: 401,69 vs 381,10 mg/dl, Relative Risk RR=1,371, 95%CI=1,081–1,856), **TM** (48,857 vs 35,404 mg/dl, RR=1,815, 95% CI=1,061–3,106), **P-C** (121,35% vs 109,56%, p<0,001, RR=1,912, 95% CI =1,083–3,378, **Hom** (14,833 vs 11,808 µmol/L, p<0,01, RR=1,130, 95% CI =1,027–1,243) and **vWf** (141,70% vs 126,24%, RR=2,538, 95 % CI = 1,032–6,913).
After comparing D and nD patients with *1 vessel disease*, risk factors were found to be **LpA** (26,452 vs 11,023 mg/dl, p<0,01, RR=3,491, 95% CI =1,023–13,209), **TM** (43,40 vs 29,94 mg/dl, RR=2,624, 95%CI=1,126–6,407) and **vWf** (153,38% vs 122,46%, RR=2,212 95%CI=1,032–4,301). For patients of D and nD group with *double and triple vessel disease*, risk factors were found to be **TM** (71,301 vs 33,177 mg/dl, RR=3,436, 95% CI =1,232–9,615), **PAI-1** (54,384 vs 39,882 ng/ml, RR=1,873, 95 % CI = 1,096–3,325) **ATIII** (0,416 vs 0,309 mg/ml, RR=3,268, 95%CI=2,004–5,348 and **Fg** (411,60 vs 368,17 mg / dl, RR=1,615, 95 % CI=1,061–2,717), **P-C** (124,83 % vs 110,94%, RR=3,205, 95%CI=1,139–9,009) and **UA** (7,068 vs 5,489, RR=2,941, 95%CI=1,024–8,638) respectively.
**Conclusion**: Several risk factors (LpA, homocysteine, thrombomoduline, vW factor, AT-III, fibrinogen, proteins C and AI-1, uric acid) seem to associate with coronary heart disease in patients with diabetes. The role of emerging risk factors in routine screening for risk prediction and strategy management remains to be established.

## 1163

**LDL-cholesterol reduction is associated with a decrease in plasma C reactive protein (CRP) in non-smoking but not in smoking Type 2 diabetic patients**
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**Background and aims:** Sub-clinical inflammation favours the development of diabetes cardiovascular complications: it is possible that the risk reduction associated with treatment of traditional cardiovascular risk factors is due in part to an associated reduction of the pro-inflammatory state. Cigarette smoking is a major pro-inflammatory factor, and it is possible that smoking could limit the potential favourable effects of risk factor treatment. (for instance of reducing LDL cholesterol levels) on inflammatory markers such as CPR plasma levels.
**Materials and methods:** To test this hypothesis, 48 type 2 diabetic subjects, without any clinical and/or instrumental sign of cardiovascular disease , 32 non-smokers (NS) (age 60,2 ± 5,3 yrs, BMI 31,4 ± 4,2 Kg/sq. m., diabetes duration 7,9 ± 5,3 yrs, HbA1 c 7,± 1) and 16 smokers (S) (age 60,5 ± 5,7 anni, BMI 27,6 ± 2,4 Kg/sqm, diabetes duration 8,8 ± 6,6 yrs, HbA1 c 7,5 ± 1,4%, smoking on average since 40,6 yrs an average 20 cigarette/day) underwent a treatment (diet or diet+statins) aimed at reducing LDL cholesterol levels to the targets suggested by ATP III guidelines. Before and after treatment., HbA1 c, LDL cholesterol and plasma CPR were measured and compared.
**Results:** HbA1 c levels decreased slightly and non significantly in both groups (NS=7,3 ± 0,8 % vs 7,7 ± 1,0%, p= n.s., S=7,1 ± 0,7% vs 7,5 ± 1,3%, p= n.s.). LDL cholesterol values, on the contrary, decreased significantly after treatment in both groups (NS= 112 ± 35 mg/dl vs 140 ± 36 mg/dl, p<0.01, S= 107 ± 23 mg/dl vs 141,4 ± 35 mg/dl, p<0.01). However, while in Non Smokers LDL cholesterol decrease was associated with a significant reduction in plasma CRP levels (4,5 ± 1,5 mg/l vs 6,7 ± 1,6 mg/l , p<0.05), in Smokers CRP values were virtually identical before and after treatment (7,7 ± 2,6 mg/l vs 7,6 ± 2,3 mg/l p= ns).
**Conclusion:** Our data suggest that in type 2 diabetic subjects improvement in LDL cholesterol level ameliorates the pro-inflammatory condition of these patients, as reflected by a decrease in CRP plasma levels. The absence of this effect in smokers suggests that the presence of a strong pro-inflammatory stimulus, such as smoking, prevents the occurrence of the positive effect of LDL cholesterol lowering on the inflammatory state and indicates an additional possible mechanism for the reported less effectiveness in smokers of treatment of traditional cardiovascular factors

## 1164

**Rosiglitazone reduces novel biomarkers of cardiovascular disease in subjects with Type 2 diabetes mellitus already on statin therapy**
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**Background and aims:** Cardiovascular disease (CVD) is the major cause of morbidity and mortality in type 2 diabetes (T2DM). Increased preponderance of small dense LDL particles and decreased HDL are atherogenic factors associated with insulin resistance (IR) and the Metabolic Syndrome. IR is also associated with hypercoagulable and proinflammatory states, which increase CVD risk. In T2DM, treatment with rosiglitazone (RSG) has demonstrated conversion of the small dense LDL Type B phenotype (LDL Rf < 0.263) to the less atherogenic Type A phenotype (LDL Rf ≥ 0.263), as well as improvement in concentrations of additional CVD biomarkers including C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI-1) and matrix metalloproteinase-9 (MMP-9). The effect of RSG on CVD risk factors in T2DM subjects on statin therapy with predominately small dense LDL was evaluated.
**Materials and methods:** Seventy two subjects on diet/exercise, or metformin monotherapy, who had received at least 8 weeks of statin therapy, and had LDL Rf < 0.263 were randomized to the addition of placebo (n = 14) or RSG 4 mg/day (n = 29) or 8 mg/day (n = 29).
**Results:** By study end (week 12), 24% (4 mg/day) and 36% (8 mg/day) of patients on RSG had converted to a pattern of predominately large buoyant LDL Rf (LDL Rf ≥ 0.263) compared to no conversion in the placebo group. Significant reductions in CRP and PAI-1 were observed in RSG-treated subjects.

| | Placebo (n = 14) | RSG 4mg/day (n = 29) | RSG 8mg/day (n = 29) |
|---|---|---|---|
| % patients converted to LDL Rf ≥ 0.263 (%) | 0 | 23.5 | 36* |
| Mean change & % change (95% CI) from baseline: | | | |
| LDL Rf | -0.0003 (-0.0112, 0.0107) | 0.011* (0.0033, 0.0187) | 0.014* (0.0069, 0.0212) |
| CRP (%) | 17.26 (-18.41, 68.50) | -35.38* (-49.67, -17.05) | -39.08* (-52.36, -22.10) |
| PAI-1 antigen (%) | -22.69 (-42.12, 3.28) | -10.63 (-26.81, 9.13) | -22.65* (-37.05, -4.96) |
| PAI-1 activity (%) | -29.02 (-57.08, 17.38) | -17.30 (-41.45, 16.83) | -30.72* (-51.14, -1.76) |
| MMP-9 (%) | 20.13 (-14.59, 68.97) | -8.70 (-27.39, 14.79) | -17.76 (-34.44, 3.17) |

* Statistically significant ($P < 0.05$)

**Conclusions:** In T2DM subjects on statins, RSG significantly increased LDL particle size and reduced CRP and PAI-1, suggesting RSG may reduce CVD events.

## 1165

**An eighteen-month trial comparing the effects of gliclazide MR and glibenclamide on atherogenic risk markers in Type 2 diabetes**
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**Background and aims:** Gliclazide has been demonstrated, in vitro or in short-term clinical studies of type 2 diabetic patients, to improve various biochemical parameters linked to atherogenous risk. The aim of this study was to compare the long-term effects of gliclazide Modified Release (MR) and glibenclamide on carotid intima-media thickness (IMT) and serum markers of atherosclerosis in patients with type 2 diabetes.
**Materials and methods:** A total of 46 patients with type 2 diabetes without clinical macrovascular complications, aged (40–69 yr), with a mean HbA1 c of 7.2%, were included in a double-blind, parallel, randomised study to receive either gliclazide MR (30–120 mg) or glibenclamide (5–20 mg) for 18 months. Carotid IMT, serum levels of soluble endothelial adhesion-molecules (sICAM, sE-Selectin and sVCAM) and C-reactive protein (CRP) were

measured at 0, 6, 12 and 18 months. Analysis of covariance adjusted for baseline values was made for comparison between treatment groups and paired t-test was used for intra-group comparison. Results are expressed as mean ± SD in the Full Analysis Set.
**Results:** $HbA_{1c}$, serum lipids, blood pressure and body mass index were well-controlled and stable throughout the study without significant difference between the two groups. Carotid IMT was within normal range at baseline and did not change during the 18 months. However, patients in the gliclazide MR group (n=19) showed significant reductions of sICAM (from 333 ± 104 to 288 ± 84 ng/ml vs 326 ± 104 to 333 ± 106 ng/ml in the glibenclamide group (n=22), p = 0.0076) and sE-Selectin (from 67 ± 31 to 51 ± 30 ng/ml, p < 0.0001 vs 55 ± 24 to 54 ± 27 ng/ml in the glibenclamide group (p=NS)). VCAM was not significantly modified in either group. There was also a trend towards significant reduction of CRP with gliclazide MR (from 4.16 ± 4.14 to 3.48 ± 3.77 ng/ml, p = 0.0755, vs 3.85 ± 2.92 to 4.11 ± 2.8, p = 0.56 in the glibenclamide group).
**Conclusion:** In patients with type 2 diabetes, changes in IMT and serum atherogenic markers are not necessarily concordant. Gliclazide MR as compared to glibenclamide led to significant reductions in serum levels of sICAM and sE-Selectin and a trend towards reduction of CRP. Therefore, gliclazide MR may have an antiatherogenic potential by reducing early serum markers of atherosclerosis.
*This is an investigator's initiated study supported by Servier.*

# PS 119

# Insulin resistance: metabolic syndrome and cardiovascular disease

## 1166

**The metabolic syndrome is independently and gradually predictive for vascular events both in diabetic and in non-diabetic coronary patients**
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**Background:** The metabolic syndrome strongly increases the incidence for cardiovascular disease. However, data on the metabolic syndrome in patients already affected by coronary artery disease are scarce.
**Methods:** We enrolled 750 patients referred for coronary angiography. According to ATP III criteria, the metabolic syndrome was defined as the presence of any three of: waist circumference >102 cm in men and >88 cm in women, triglycerides ≥150 mg/dl, HDL cholesterol <40 mg/dl in men and <50 mg/dl in women, blood pressure ≥130/≥85 mmHg, or fasting glucose ≥110 mg/dl. The incidence of vascular events was recorded during a mean follow-up period of 2.3 ± 0.4 years.
**Results:** Among patients with diabetes mellitus type 2 (n = 164) the prevalence of the metabolic syndrome was higher (68.3% vs. 28.7%; p <0.001) than among nondiabetic patients. After adjustment for age, gender, smoking, and LDL cholesterol the metabolic syndrome proved independently predictive for vascular events both among patients with (2.980 [1.024 - 8.670]) and without (1.721 [1.015 - 2.916]) diabetes. Cardiovascular risk increased with an increasing number of components of the metabolic syndrome (p for trend <0.001). Compared with patients without any component of the metabolic syndrome, adjusted odds ratios for patients with one through five components were 1.818 (0.662 - 4.993), 2.046 (0.741 - 5.651), 2.235 (0.806 - 6.197), 5.100 (1.832 - 14.193), and 5.566 (1.728 - 17.930), respectively.
**Conclusions:** Among coronary patients both with and without diabetes the metabolic syndrome is independently predictive for vascular events. The risk of vascular events increases with an increasing number of components of the metabolic syndrome.

## 1167

**Metabolic syndrome and insulin-resistance as predictors of silent myocardial ischemia in apparently uncomplicated Type 2 diabetic patients**
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**Background and aims:** Metabolic syndrome is associated with elevated morbidity and mortality for overt coronary artery disease (CAD). The clinical onset of CAD is often silent in diabetic patients, and this evidence represents a further dangerous clinical condition in the progression of vascular disease. Since the relationship between metabolic syndrome and silent CAD was never studied, we investigated several potential mechanisms associated with the metabolic syndrome and silent CAD in Type 2 diabetic patients.
**Materials and methods:** We evaluated the prevalence of metabolic syndrome in 169 uncomplicated Type 2 diabetic out-patients with angiographically verified silent CAD and in 158 Type 2 diabetic patients without myocardial ischemia at exercise ECG, 48-hours ambulatory ECG and stress echocardiography. The groups were matched for sex, age, glycemic control and diabetes duration. Metabolic syndrome was defined according to the National Cholesterol Education Program criteria. $HOMA_{IR}$ was calculated to estimate insulin-resistance in diabetic patients treated with diet alone or with oral agents (122 CAD patients and 115 NO CAD patients).
**Results:** The prevalence of metabolic syndrome (59.8% versus 44.3%; p=0.0052) and $HOMA_{IR}$ levels (5.4 ± 2.1 versus 4.9 ± 2.8; p=0.0446) were significantly higher in CAD than in NO CAD Type 2 diabetic patients. Multiple logistic regression analysis showed that metabolic syndrome was significantly associated with silent CAD (Odds ratio: 2.44; 95% CI: 1.19–5.02;

p = 0.015). Among patients on diet alone or oral agents $HOMA_{IR}$ was a strong predictor of silent CAD (Odds ratio: 9.56; 95% CI : 2.49–36.72; p=0.001).
**Conclusion:** Taken together, our data show an independent association of both metabolic syndrome and insulin-resistance with silent CAD in Type 2 diabetic patients. Therefore, the clinical eterogeneity of Type 2 diabetes could also include silent CAD, so suggesting a common molecular mechanism in the pathogenesis of CAD and metabolic syndrome in diabetes mellitus. However, other studies will be useful in order to establish whether metabolic syndrome and $HOMA_{IR}$ should be reliable markers to identify diabetic patients for further screening of silent CAD.
*Supported by: Grant Maugeri Foundation 2004*

## 1168

**Prevalence and incidence of micro- and macro-vascular diseases among Type 2 diabetic patients with and without metabolic syndrome**
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**Background and aims:** Patients with diabetes are at increased risk for cardiovascular disease (CVD) compared to those without diabetes. The presence of metabolic syndrome (MS) in these patients may further increase the risk. This study aimed to assess the prevalence and incidence of micro- and macro-vascular diseases among type 2 diabetic patients with and without MS in a French cohort of patients.
**Materials and methods:** In 2002, 135 French general practitioners and specialists were surveyed to collect information on a random sample of their patients with type 2 diabetes. Information collected includes patient demographics, treatment patterns, and the prevalence and 6-month incidence of micro- and macro-vascular complications. MS was defined according to an adapted version of the WHO criteria (≥ 2 of the following abnormalities in addition to diabetes): BMI > 30 kg/m$^2$ or on drug treatment for obesity; serum triglycerides level ≥ 1.7 mmol/L, HDL cholesterol level < 0.9 mmol/L in men or < 1.0 mmol/L in women, or on fibrates; blood pressure ≥ 140/90 mmHg or on hypotensive drugs; or microalbuminuria/proteinuria/ESRD/nephropathy. The association between prevalence and incidence of macro- and micro-vascular events and MS were assessed by the Mantel-Haenzel $\chi^2$ test and multi-variable logistic regression.
**Results:** Among 3345 type 2 diabetic patients who had complete data, 56% had 2 or more MS conditions besides diabetes (therefore met WHO MS definition), 18% had one MS condition, and the remaining 24% did not have any additional MS condition. Patients with MS had longer mean duration of diabetes (8.9 vs. 7.9 years, $p < 0.0001$, Wilcoxon test) and higher mean $HbA_{1c}$ level (7.8 vs. 7.4%, $p < 0.0001$, Wilcoxon test). The prevalence of macro-/micro-vascular events among type 2 diabetic patients who had two, one and 0 additional MS conditions were 31%/34%, 22%/17% and 17%/12% respectively (p<0.001, Mantel-Haenzel $\chi^2$ test). The 6-month incidence of coronary heart disease (CHD) among patients with and without MS was 7.7% and 3.2% respectively (p<0.001, $\chi^2$ test). After adjusting for demographics and co-morbid conditions, the likelihood of having ≥ 1 CHD events in the previous 6 months was significantly higher among patients with MS (Odds Ratios = 1.98, 95% CI 1.37 – 2.86, $p < 0.0005$).
**Conclusion:** Among patients with type 2 diabetes, the presence of MS conditions further increases the risk of micro- and macro-vascular diseases. These patients may require closer clinical attention and more intensive management to reduce micro- and macro-vascular complications.

## 1169

**Relationship between decreased insulin sensitivity and cholesterol metabolism impairments in Type 2 diabetics and nondiabetic subjects with angiographically verified coronary artery disease**
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**Background and aims:** It has been previously shown that in nondiabetic patients decreased insulin sensitivity has been associated with variety cardiovascular risk factors, including dyslipidemia. In contrast, the relationship between impairments in insulin sensitivity and the changes in cholesterol metabolism relevant for the pathogenesis of coronary artery disease (CAD) in patients with type 2 diabetes has not yet been elucidated. Therefore, the aim of this study was to evaluate insulin sensitivity impairment, i.e. insulin resistance levels and their relationship to total cholesterol (Ch), HDL-Ch, LDL-Ch and triglyceride (Tg) levels, as well as Tg/HDL ratio reflecting small dense LDL levels in 30 patients with type 2 diabetes and CAD (group A), 30 patients with type 2 diabetes without CAD (group B), 45 nondiabetics with CAD (group C) and 45 nondiabetics without CAD (group D).
**Materials and methods:** Insulin resistance was determined by homeostasis model assessment (HOMA-IR) and total, HDL-Ch, LDL-Ch and Tg levels by chromatography. CAD was angiographically verified in each patient included in this study and defined as a stenosis with narrowing of the lumen >50% with respect to the pre-stenotic segment in the following coronary blood vessels: main stem, left descending, circumflex and right coronary artery. Absence of CAD was determined when the narrowing of the lumen was < 10% at the respective arteries. Patients with intermediary degree of stenosis (between 10 and 50%) were excluded from the study.
**Results:** We found that HOMA-IR values were significantly higher in group A compared to group B (9.54+/–1.16 vs 4.36+/–0.97; p<0.01) and in group C compared to group D (3.93+/–0.47 vs 1.79+/–0.18; p<0.01). Simultaneously, similar pattern of changes was found with total Ch, LDL-Ch, Tg levels (total Ch: A: 6.5+/–0.5; B: 5.7+/–0.3; C:6.3+/–0.3; D:5.6+/–0.2; LDL-Ch: A: 4.5+/–0.5; B: 4.0+/–0.4; C:4.4+/–0.2; D:3.8+/–0.3; Tg: A: 2.5+/–0.2; B: 2.0+/–0.1; C:2.2+/–0.2; D:1.5+/–0.2 mmol/l; A vs B and C vs D p<0.05, respectively) and Tg/HDL ratio (A:2.91+/–0.49 vs B:2.02+/–0.05; p<0.05; C:2.29+/–0.27 vs D:1.56+/–0.29; p<0.05), while HDL-Ch levels were significantly lower in group A vs group B and in group C vs group D (A:0.98+/–0.06 vs B:1.24+/–0.14; p<0.01; C:1.02+/–0.05 vs D : 1.46 + / – 0.15 mmol / l; p<0.001). However, we found that the increased HOMA-IR correlated significantly in type 2 diabetics with the changes in Tg/HDL ratio (r=0.531; p<0.05), Tg (r=0.356; p<0.01) and HDL-Ch levels (r=–0.565; p<0.05) and in nondiabetics they correlated with the changes in Tg/HDL ratio (r=0.379; p<0.05) and Tg levels (r=0.383; p<0.01).
**Conclusion:** Our results signify that both in type 2 diabetic patients and nondiabetic subjects, insulin resistance, as an important determinant of CAD, is strongly associated with increased Tg/HDL ratio reflecting increased small dense LDL levels. The results imply that Tg/HDL ratio might be a useful marker for evaluation of atherogenic influence of insulin resistance regarding CAD both in type 2 diabetes and nondiabetics.

## 1170

**In obese subjects with the metabolic syndrome, cardiac autonomic dysfunction is associated with higher blood pressure and glycemic levels**
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**Background and aims:** We have previously shown that obesity is associated with a high rate of abnormal heart rate variability (HRV). Hypertension is often found in patients with the metabolic syndrome (MS). The aim of the present study was to look for an association between MS and cardiac autonomic dysfunction (CAD) in a series of nondiabetic obese subjects and to determine whether the MS is more severe in patients with CAD.
**Materials and methods:** We included 195 obese subjects free of diabetes according to an oral glucose load and without anti-hypertensive treatment. HRV was analyzed during three standardized tests (Valsalva, lying-to-standing and deep breathing).
**Results:** CAD was defined by at least one abnormal test and found in 92 subjects. MS, defined by the NCEP criteria, was found in 71 subjects. The prevalence of CAD did not differ significantly between those with and those without MS (40.8% vs 50.8%). Among the patients with MS, those with CAD (n = 29) had higher HbA1c (p = 0.05) and fructosamine (p = 0.025) levels and higher systolic blood pressure (p = 0.025) than those with normal HRV tests (n = 42) whereas BMI and waist circumference were not significantly different. Among the patients free of MS, those with CAD (n = 63) had significantly lower HDL-cholesterol levels (p < 0.0001) but not higher blood pressure than those with normal HRV (n = 61).
**Conclusion:** In conclusion, 1) in this series of nondiabetic obese subjects the presence of MS is not associated with a higher rate of CAD, 2) abnormal HRV which results mainly from depressed vagal activity may contribute to a rise in blood pressure and the onset of diabetes, possibly through an increase in sympathetic activity.

## 1171

**"Hypertensive waist" as a cardiovascular risk factor in newly diagnosed Type 2 diabetes persons**

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**Background:** Both metabolic syndrome and Type 2 Diabetes are associated with high cardiovascular risk, due to the cluster of traditional cardiovascular risk factors. In daily practice, there are emerging cardiovascular risk factors, such as large waist, that can be important for the screening and assessment of cardiovascular risk.
**Aims:** The aim of this analysis was to evaluate the role of hypertensive large waist in cardiovascular risk screening and assessment in persons with newly diagnosed Type 2 diabetes.
**Materials and methods:** The analysis has included 1152 persons newly diagnosed with Type 2 diabetes in 2003, women 53.9% and men 46.1 %. Demographic, clinical and biochemical parameters have been recorded. Cardiovascular risk has been assessed according to the European Guidelines on Cardiovascular disease of the Third Joint Task Force of European and other Societies, for a period of ten years and high risk European regions. "Hypertensive waist", assessed according to ATP III, has been used as cutoff point to compare the cardiovascular risk.
**Results:** The couple of "hypertensive waist" was present in 79 % of men and 77.8 % of women. The couple of "hypertriglyceridemic waist" was present in 41.6 % of men and 32.2 % in women. The mean cardiovascular risk of the entire group was 3.44 (± 3.49). In both men and women, cardiovascular risk was significantly associated with hypertensive waist ($p<0.0001$ and $p< 0.05$).
**Conclusion:** The most frequent pathologic association in newly diagnosed Type 2 diabetes persons is hypertensive waist. Hypertensive waist should be considered the first tool for the screening and assessment of cardiovascular risk in high risk persons.

## 1172

**Insulin resistance is an independent predictor of vascular events in diabetic patients with coronary artery disease**

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**Background:** Patients with both diabetes and established coronary artery disease are at a high risk of cardiovascular events. Insulin resistance (IR) is a central feature of diabetes mellitus type 2 (DM2). Therefore, the impact of IR on the incidence of vascular events in diabetic patients with established CAD is of particular interest.
**Methods:** We estimated insulin resistance by the HOMA index in 495 patients with angiographically proven CAD and recorded the incidence of vascular events over a mean follow-up time of 2.3 ± 0.4 years.
**Results:** The HOMA index was higher in coronary patients with DM2 (n = 127) than in nondiabetic coronary patients (6.5 ± 5.9 vs. 3.0 ± 4.2; $p <0.001$). Thirty-one (23.8%) patients with DM 2 and 60 nondiabetic patients (14.5%) experienced at least 1 vascular event. In Cox regression analysis adjusting for age, gender, and baseline extent of coronary artery disease (number of angiographic stenoses ≥50%) diabetes was an independent predictor for the incidence of vascular events (OR = 1.725 [1.116 – 2.667]; p = 0.014). Equally, the HOMA index proved independently predictive for the incidence of vascular events in the total study cohort: the standardized OR adjusted for age, gender, and baseline extent of CAD was 1.178 [1.026–1.351]; p = 0.010. In subgroup analyses with respect to diabetes status, the HOMA index was significantly predictive for vascular events in patients with diabetes (OR = 1.354 [1.083 – 1.694; p = 0.008]), but not among nondiabetic patients (OR = 1.022 [0.729 – 1.432]; p = 0.901).
**Conclusions:** In the setting of secondary prevention, IR is a strong and independent predictor of vascular events among patients with DM2. Thus, the degree of IR significantly contributes to the adverse effects of diabetes on the prognosis in coronary patients.

## 1173

**Adiponectin in chronic heart failure – relation to insulin resistance and markers of endothelialdysfunction and oxidative stress**

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**Background and aims:** Chronic heart failure is characterised by insulin resistance (IR), but the mechanism behind the heart failure mediated IR is unknown. The adipocytokine adiponectin (ADPN) has been related to the pathogenesis of IR and to endothelial dysfunction. The aims of this study were to examine whether ADPN is involved in IR in chronic heart failure, and to evaluate the association between ADPN and markers of endothelial dysfunction and oxidative stress.
**Material and methods:** A prospective study including 195 consecutive patients with chronic heart failure, recruited from our specialised heart failure clinic. Mean age was 69.3 (± 10.2) years, 78 % was male, mean BMI was 27. 3 (± 5.4), range: 15.5– 41.2 kg/m$^2$, and mean left ventricular ejection fraction was 30 (± 8) %. A total of 26 % had DM by definition, and 55 % had ischemic heart disease (IHD). Severity of heart failure was assessed by measuring the cardiac marker N-terminal pro brain natriuretic peptide (NT-proBNP). Insulin resistance was determined by fasting plasma insulin and glucose, as the HOMA-IR index. Samples for measurement of ADPN, soluble E-selectin (an index of endothelial activation) and oxidised LDL cholesterol (oxLDL) were obtained.
**Results:** After adjustment for HOMA-IR and BMI, low ADPN levels was associated with presence of DM (p = 0.022), whereas no significant association with IHD was found (p = 0.47). In unvariate analysis plasma ADPN was inversely correlated with BMI ($r=-0.43$, $p< 0.0001$), fasting insulin ($r=-0.40$, $p<0.0001$), HOMA-IR ($r=-0.24$, $p<0.001$) as well as E-selectin ($r=-0.13$, $p=0.033$) and oxLDL ($r=-0.18$, $p=0.012$). Furthermore, ADPN was positively correlated with HDL ($r=0.56$, $p<0.0001$) and with NT-proBNP ($r=0.39$, $p<0.0001$). In a multivariable linear regression analysis for the total population, NT-proBNP ($r=0.29$, $p<0.001$) and HDL (0.46, $p<0.001$) were positive independent predictors of ADPN while oxLDL ($r=-0.20$, $p=0.028$) and fasting insulin ($r=-0.18$, $p=0.038$) were inversely independent predictors of plasma ADPN levels, after adjustment for age, gender, BMI, lipids, creatinine clearance, presence of DM or IHD, as well as the use of ACE-inhibitors and beta-blockers. The main predictor of IR in DM was NT-proBNP ($r=-0.42$, $p=0.028$) with no independent correlation with ADPN, whereas in the non-DM both plasma ADPN and BMI were independent predictors of IR ($r=-0.46$, $p<0.0001$ and $r=0.45$, $p<0.0001$, respectively). This indicates that plasma ADPN levels are associated with IR in patients with chronic heart failure.
**Conclusion:** Low plasma ADPN levels are independently associated with increased IR and oxidative stress in patients with chronic heart failure. Furthermore, plasma ADPN was independently and positively related to the cardiac peptide NT-proBNP. This unexpected finding could partially be explained by the possible elimination of NT-proBNP in adipose tissue by a specific clearance receptor.

## 1174

**Coronary heart disease experience and time to insulin with rosiglitazone-metformin combination in overweight and obese patients with Type 2 diabetes in the UK**

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**Background & aims:** Coronary heart disease (CHD) is a major cause of morbidity and mortality in Type 2 diabetes. Insulin insensitivity and beta cell dysfunction, the metabolic defects underlying the condition, appear closely related to CHD risk factor profiles. We assess the impact of Rosiglitazone in combination with Metformin for treatment of Type 2 diabetes patients when Metformin monotherapy fails to maintain glycaemic control on CHD experience and time to insulin compared to conventional care of adding Glibenclamide (SU) to failing Metformin monotherapy.
**Methods:** DiDACT, an established long-term model of Type 2 diabetes in the UK, represents the natural progression of Type 2 Diabetes through a novel metabolic core, which generates sets of dynamic risk factor trajectories consistent with pre-specified therapeutic strategies. These link into the main module to project long-term diabetes-related morbidity and mortality. UKPDS risk estimation algorithm is used to model CHD morbidity and mortality. The algorithm includes direct mechanisms for hyperglycaemia, hypertension and dyslipidaemia to influence CHD. We simulated treatment

histories for mixed incident cohorts of 1000 newly diagnosed overweight (mean BMI=26 kg/m²) and obese patients (mean BMI=34 kg/m²). Following failure of glycaemic control with Metformin monotherapy, combination therapy adding Rosiglitazone (4 mg twice daily) was compared to adding titrated SU. The threshold for switching therapies was 7% HbA1 c, the midpoint of the range recommended by the National Institute for Clinical Excellence (NICE).
**Results:** The model predicts that adding Rosiglitazone to Metformin produces better glycaemic control ($HbA_{1c}$) and improves SBP in most patients. Rosiglitazone delays the onset of insulin by between 4.5 and 6 years, which is a good indicator of superior glycaemic control. Appropriate prescribing of lipid regulating therapy ensures that dyslipidaemia risk is equalised across treatment strategies. Improvements in $HbA_{1c}$ and SBP amongst Rosiglitazone patients lead to reductions in CHD incidence and prevalence rates. Reductions in incidence rates occur during Rosiglitazone treatment. Prevention of CHD events at this relatively early stage of diabetes progression contributes to improved survival. Estimated benefits are conservative as some patients suffer metabolic crisis, comprising sudden loss of beta cell function, rapid weight loss and uncontrollable rise in glycaemia, necessitating oral therapy termination, bypassing Rosiglitazone and jumping straight to insulin therapy. Moreover, improved survival with Rosiglitazone therapy increases total lifetime CHD risk exposure.
**Conclusion:** In addition to reduced microvascular complications and insulin delay, Rosiglitazone in combination with Metformin leads to reductions in both experience of and exposure to CHD when compared with conventional care of Metformin in combination with SU in overweight and obese patients in the UK.

CHD Incidence and Prevalence at termination of Rosiglitazone therapy

| Overweight | Total Life Years | Insulin Initiation (Years) | Patients entering state (Incidence) | Life-years in state (Prevalence) |
|---|---|---|---|---|
| **Metformin + SU** | 13,061 | 11 | 176 (17.6%) | 3,275 (25.1%) |
| **Metformin + Rosiglitazone** | 13,170 | 17 | 158 (15.8%) | 3,269 (24.8%) |
| **Obese** | | | | |
| **Metformin + SU** (18.4%) (25.2%) | 12,998 3,278 | 11 | 184 | |
| **Metformin + Rosiglitazone** | 13,095 | 15.5 | 168 (16.8%) | 3,273 (24.9%) |

*Financial support was provided by GlaxoSmithKline*

## 1175

**A lack of acute synergistic interaction between insulin and pioglitazone on vasodepressor responses in aorta from Type 2 diabetic rats**
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**Background and aims:** Thiazolidinediones have some direct vascular effects independent from their insulin-sensitizing effects. For ex., troglitazone and pioglitazone (PIO) have been shown to cause acute dilation of peripheral blood vessels. On the other hand, besides its key role in the regulation of carbohydrate metabolism, insulin (INS) has important cardiovascular actions including acute vasodilation. Vasodilator/vasodepressor effect on INS is blunted in diabetes. Therefore, we evaluated whether PIO directly modulates the vasodepressor effect of INS, in vitro. Recently, a new experimental type 2 adult diabetic rat model has been described. We established similar type of diabetic syndrome in this study and we tested whether PIO acutly augments the vasodepressor action of INS, hence it may increase INS utilisation during the treatment of type 2 diabetic state.
**Materials and methods:** Diabetes was induced by streptozotocine (65 mg / kg) + nicotinamide (230 mg/kg), i.p., in male adult Wistar rats. Blood glucose, HbA1 c, plasma INS levels, blood pressure (BP) (by tail-cuff method) were measured. After 16 weeks, aortae from diabetic (D) and age-matched non-diabetic (ND) rats were removed. Endothelium-denuded rings were mounted in organ baths for isometric tension recordings. Cumulative concentration-response curves of serotonin (5-HT) were evaluated before and after one hour incubations with INS (0.1 μU / l: physiologic concentration or 100 mU/ml: supraphysiologic concentration), or PIO (10 μM), or INS + PIO (the same concentrations) in D aortae. In some rings, KCL- and BayK 8644- induced contractions were evaluated in the presence or absence of PIO for one hour. $pEC_{50}$ values (-log $EC_{50}$) were calculated by lineer regression analysis of the curves and taken as a measure of the sensitivity of aortae to the agonists. Statistical differences were evaluated by Student's t-test at .05 probability level.
**Results:** D rats showed mild hyperglycemia (fed: ND, 109 ± 7 vs. D, 202 ± 9 mg/dl, n:10, $p<.05$). There was no significant differences in INS levels between the groups (ND, 35.1 ± 5.3 vs. D, 30.3 ± 5.1 μU/ml, n:10, $p>.05$). HbA1 c levels were higher in D rats (ND, 4.1 ± 0.05 vs. 6.0 ± 0.1 %, n:10, $p<0.05$). BP values were similar in both groups. In pharmacodynamic studies, INS (100 mU/ml) and PIO, each alone, but not 0.1 μU/l INS, significantly depressed maximum contraction (Emax) induced by 5-HT by 40 ± 4 % and 30 ± 3 % respectively (n:5, $p<.05$). pEC value for 5-HT was also decreased markedly. Vasodepressor effect of INS was found to be increased when used in combination with PIO (n:5, $p<.05$), but no further significant depression was observed compared to PIO incubation alone. Emax values induced by $K^+$ depolarization or by BayK were inhibited partly after one hour PIO incubations in D rings (n:5, $p<.05$). On the other hand, 5-HT, KCl and Bay K-induced contractions were increased significantly in D aortae when compared to ND aortae (n:5, $p<.05$).
**Conclusion:** The results demonstrate a lack of an acute synergistic interaction between INS and PIO in the reactivity of type 2 diabetic rat aorta. However, the acute vasodepressor effect of PIO, partly by $Ca^{2+}$ channel inhibition, may be beneficial by improving INS utilization due to increasing blood flow to the INS-sensitive tissues in type 2 diabetes.

# PS 120

# Cardiac complications: blood flow and risk factors

## 1176

**Systolic blood pressure response after exercise in Type 1 diabetes families compared with healthy control subjects**

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**Background and aims:** We have reported evidence of increased biomarkers of oxidative stress in families of patients with type 1 diabetes. Our purposes were to compare the responses to exercise testing in control subjects and type 1 diabetes (T1DM) families, and to investigate whether exercise testing has any relationship with traditional and non cardiovascular risk factors.
**Materials and methods:** We studied 38 T1DM patients (age 37 ± 12 y; disease duration 18 ± 11 y; 10 patients with retinopathy and 9 patients with nephropathy), 76 first-degree relatives of T1DM patients (50 ± 13 y; 44 parents and 32 siblings), and 95 healthy subjects (44 ± 13 y) without established coronary heart disease who underwent routine clinical cycle ergometer test. Measurements included standardised medical history, ETT (cycling), and assessment of cardiovascular risk (body weight, blood pressure, cigarette smoking, physical activity, fasting plasma glucose and insulin, HbA1 c, plasma lipids, C-reactive protein, fibrinogen, folate, homocysteine, and plasma thiols as biomarkers of protein oxidation).
**Results:** No patient presented exercise-induced angina. Compared with age-gender-matched control subjects, T1DM patients achieved a higher maximal exercise systolic blood pressure (SBP 191 ± 30 vs 176 ± 24 mmHg, $p<0.05$). SBP remained high at 2 (145 ± 27 vs 133 ± 19, $p<0.01$) and 4 minutes (131 ± 23 vs 119 ± 14, $p<0.05$) into recovery. An attenuated heart rate response to exercise, a measure of chronotropic incompetence (82 ± 9 vs 89 ± 9%, $p<0.01$), was observed. Relatives showed higher values of systolic blood pressure at peak exercise (197 ± 27 vs 187 ± 30, $p<0.05$). In type 1 diabetic subjects, exercise-induced increase in SBP relative to maximum exercise capacity (ΔExSBP/W) was positively associated only with disease duration (R 0.7, $p<0.001$, t-value 5.0). In relatives and controls, ΔExSBP/W correlated (multiple R 0.4, $p<0.001$) positively with plasma total cholesterol (t-value 3.1) and insulin resistance estimated by homeostasis model assessment (HOMAIR) (1.9), negatively with plasma thiols (–2.0) and regular exercise (–2.3).
**Conclusion:** An abnormal blood pressure response has been identified not only in type 1 diabetic probands, but also in asymptomatic normotensive non-diabetic relatives, in which it was associated with indices of insulin resistance and oxidative damage.

## 1177

**Coronary flow reserve in the early stage of Type 1 diabetes mellitus**

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**Background and aims:** Several studies have shown a reduced coronary flow reserve (CFR) in diabetic patients without coronary artery disease, indicating an impaired ability of coronary microcirculation to adapt to increasing demand. The reduction of CFR has been demonstrated in patients with either type of diabetes and evidence of microvascular complications (autonomic neuropathy and retinopathy). How early abnormalities of coronary microcirculation occur in the course of diabetes is still unsettled. The study was performed to evaluate CFR in young normotensive type 1 diabetic patients free of microvascular and macrovascular complications.
**Materials and methods:** Eight Type 1 diabetic patients (age 27 ± 3 y, BMI 24 ± 1 kg/m$^2$ , duration of diabetes less than 5 years, HbA1 c 8.0 ± 0.5 % and nine normal subjects (age 27 ± 1 y, BMI 25 ± 1Kg/m$^2$) participated in the study. The two groups were similar for blood pressure and lipid profile, and no subject had positive family history of coronary heart disease. Diabetic patients had normal exercise treadmill test and no evidence of microvascular complication and/or autonomic neuropathy. Blood flow velocity in left anterior descending coronary artery was measured at rest and after dipyridamole (0,56 mg/Kg over 4 min) using transthoracic color-guided pulsed Doppler echocardiography. CFR was defined as the ratio of dipyridamole-induced coronary peak diastolic to resting peak diastolic flow velocity. CFR assessment was performed in the fasting state before administration of morning short-acting insulin dose.
**Results:** Fasting blood glucose was 219 ± 13 mg / dl in diabetic and 80 ± 2 mg/dl in control subjects. Resting mean blood pressure was similar in the two groups (88 ± 2 and 89 ± 2 mmHg) and remained substantially unchanged during dipyridamole infusion. Resting coronary flow velocity was significantly greater in diabetic patients (25 ± 2 cm/sec) compared with normal subjects (18 ± 0.8 cm/sec, $p=0.002$.CFR tended to be lower in diabetic patients (2.51 ± 0.18) compared with controls (2.89 ± 0.22) although the difference did not reach the statistical significance.
**Conclusion:** Resting coronary flow velocity is increased in young type 1 diabetic patients with short duration of disease whereas no significant reduction in CFR is evidenced. The elevation in basal coronary flow is in line with the finding of an increased blood flow in retinal and glomerular vascular bed.

## 1178

**Left ventricular geometry and related cardiovascular risk factors in Type 1 diabetic patients with and without nephropathy**

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**Background and aims:** Left ventricular hypertrophy (LVH) has been established as independent cardiovascular risk factor. In type 1 diabetes LVH seems to be associated with diabetic nephropathy (DN) but relation of different LVH patterns with the other risk factors remains to be clarified. We assessed the link between left ventricular geometry, 24-hour blood pressure (24-h BP), autonomic neuropathy and other cardiovascular risk factors in type 1 diabetic patients with and without DN.
**Materials and methods:** 60 patients with type 1 diabetes and normal creatinine clearance, 28 M/32 F, age 32.3 ± 9.6 (SD) years, were examined. 20 patients were normoalbuminuric (group DN0), 23 ones had microalbuminuria (group DN1) and 17 patients had proteinuria (group DN2). 15 healthy subjects were acted as control. Left ventricular mass index (LVMI) and relative left ventricular wall thickness (RWT) were determined by M-mode echocardiography. LVH was defined as LVMI >134 g/m$^2$ in men and >110 g/m$^2$ in women. 24-h BP monitoring, cardiovascular autonomic function tests, as well as hemoglobin, $HbA_{1c}$, serum cholesterol and triglycerides measurements were performed.
**Results:** LVMI and RWT was increased significantly (all $p<0.05$) in DN2 group as compared to DN1, DN0 and control group (LVMI: 124.4 ± 26.3, 102.9 ± 21.4, 100.7 ± 15.9 and 104.4 ± 16.7 g/m$^2$; RWT: 0.45 ± 0.10, 0.35 ± 0.08, 0.36 ± 0.06, 0.35 ± 0.05 respectively). The concentric type of LVH (RWT ≥0.45) was estimated in 4 DN1- and in 4 DN2-patients, the eccentric type (RWT<0.45) was found in one DN0-, in 3 DN1- and in 4 DN2-patients. So, the prevalence of LVH was significantly higher in group DN2 (47%) and DN1 (30%) as compared to group DN0 (5%). The prevalence of concentric and eccentric pattern was equal. Both left ventricular indexes correlated positively with albuminuria (LVMI: $r = 0.41$, $p = 0.01$; RWT: $r = 0.49$, $p = 0.0001$). Besides, RWT correlated with diurnal and nocturnal systolic BP ($r = 0.39$, $p=0.01$; $r=0.34$, $p=0.02$) and nocturnal diastolic BP ($r=0.40$, $p=0.01$). No correlations were found between LVMI and 24-h BP parameters. Both left ventricular indexes were higher in patients with blunted nocturnal BP reduction (non-dippers, n=33) as compared to the dippers (LVMI: 120.8 ± 24.6 vs 95.0 ± 23.1 g/m$^2$, $p=0.0001$; RWT: 0.35 ± 0.06 vs 0.31 ± 0.06, $p=0.01$). In a multiple stepwise regression analysis age, male gender and albuminuria were independently associated with LVMI ($p=0.0001$), meanwhile 24-h systolic and diastolic BP, autonomic neuropathy and hemoglobin level were predictors for RWT increasing ($p=0.008$). Cigarette smoking, $HbA_{1c}$ and serum lipids did not show associations.
**Conclusion:** The obtained results demonstrate the close relation between DN and left ventricular remodeling in type 1 diabetes. This relation can be mediated through arterial hypertension, abnormal circadian BP profile, autonomic neuropathy and anemia. Different combinations of cardiovascular risk factors cause the concentric or eccentric pattern of LVH in DN.

## 1179

**Homocysteine concentrations in Type 2 diabetic patients with silent myocardial ischemia: a predictive marker**

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**Background and aims:** Silent myocardial ischemia (SMI) is a frequent finding among diabetic patients. There is very few data on the relationship between homocysteine which is a novel cardiovascular risk factor and SMI in diabetic patients. We investigated whether plasma homocysteine have a predictive value for early diagnosis of SMI in type 2 diabetic patients
**Material and methods:** 120 diabetic patients and 25 control subjects were evaluated. Among diabetic patients 29 of them had a history or clinical signs of coronary artery disease (CAD). All other patients who had normal ECG's and no history or clinical signs of CAD were screened by exercise test. 38 patient with maximal negative exercise test were labelled as CAD (-) diabetic patient group. Angiography was performed to patients who had positive exercise test and among them 23 patients had angiographically documented SMI.
**Results:** In CAD (+) and SMI group, serum homocysteine concentrations were significantly higher than CAD (-) and control group ( 14.2 ± 6.6, 15.7 ± 7.8, 9.6 ± 3.23, 9.3 ± 2.25µmol/L respectively). In SMI (+) diabetic group there was a significant correlation between homocysteine levels and creatinine, microalbuminuria and folic acid levels. Multiple regression analysis showed that homocysteine concentration was dependent on microalbuminuria, folic acid levels and presence or absence of ischemia.
**Conclusion**: The present investigation shows an association of homocysteine with SMI in diabetic patients. Other studies are needed to establish whether homocysteine levels can be used as suitable marker for CAD screening in type 2 diabetic patients.

## 1180

**Evaluation of dobutamine stress echocardiography (DSE) and gated-single photon emission computed tomography (SPECT) in comparison to coronary angiography in detecting silent asymptomatic coronary stenoses in diabetic patients with very high cardio-vascular risk**

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**Background and aims:** Silent coronary artery disease (CAD) is frequent in diabetic patients and its detection is recommended, first by exercise stress testing (EST) and then by SPECT or DSE when EST is impossible or non conclusive. Whether SPECT and DSE are equivalent in detecting CAD in diabetic patients has never been studied. The aim of this study was to assess the efficacy of gated-SPECT coupled with EST, and dobutamine stress echocardiography (DSE) in comparison to coronary angiography on the detection of asymptomatic coronary stenoses.
**Materials and methods:** Fifty-four asymptomatic diabetic patients, with peripheral arterial disease (PAD) and/or under dialysis, but without rest ECG abnormalities, were enrolled in this prospective study. Thirty-eight of them have been submitted to the 3 procedures at the time of this preliminary report.
**Results:** Diabetic patients were 32 males and 6 females, aged 61 ± 9 years, 9 type 1 and 27 type 2, with a known duration of the disease of 13 ± 9 years, a BMI of 28 ± 5 kg/m$^2$, a HbA1 c of 7,6 ± 2,1%, 20 with PAD only, and 18 under dialysis. No serious complication occurred during the procedures. Coronary angiography was positive in 17 patients (45%): 7 out of 18 dialysed patients (39%) and 10 out of 20 PAD patients (50%). 12 patients had a 1-vessel, 3 a 2-vessel and 2 a 3-vessel disease. While the precision was the same for both procedures (71%), sensitivity, specificity, predictive positive and negative values differed: respectively 41%, 95 %, 88 %, and 67 % for DSE, and 71 %, 71 %, 67 %, and 75 % for gated-SPECT.
**Conclusion:** This is the first evaluation of both DSE and gated-SPECT in comparison to coronary angiography in asymptomatic diabetic patients. Silent coronary stenoses are very common in this high risk diabetic population, more frequent in patients with PAD (50%) than under dialysis (39%). More than half of them (53%) had a revascularisation procedure (percutaneous transluminal coronary angiography with stent). If DSE has an excellent specificity, its sensitivity is poor, leading to an unacceptable number of non diagnosed stenoses. In contrast, gated-SPECT has a better sensitivity but its specificity is lower, leading to numerous useless coronary angiographies. Such non satisfactory results justify to assess other noninvasive techniques such as noninvasive coronary angiography.
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## 1181

**Diabetes mellitus confers an increased risk of death at 1 year in subjects with elevated troponin t concentrations**

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**Background and aims:** Diabetes is a potent and independent risk factor for coronary artery disease, which is the most common and costly vascular complication of diabetes. It is a major determinant of the mortality and morbidity among subjects with diabetes. Studies suggest that diabetes adversely affects early and late outcomes post acute myocardial infarction (AMI). It increases the risk of adverse cardiovascular outcomes to a level above that of subjects without diabetes but who have established coronary artery disease. Recent data support the role of troponin t, a biochemical marker of myocardial necrosis in risk evaluation of acute coronary syndromes.
**Materials and methods:** We performed a retrospective cohort study to evaluate the outcome of subjects with and without diabetes who presented to hospital with a raised troponin t concentration and who were treated according to current UK guidelines. We collected data on all subjects presenting to our hospital with an elevated troponin t concentration over a 3-month period and observed them over the following year. We recorded the presenting troponin t concentration, venous glucose concentration, all cause mortality at 1 year and whether they represented to our hospital with elevated troponin t concentrations in the following 1 year. AMI was diagnosed when troponin t concentrations were ≥ 0.20 ng/ml. Concentrations between 0.03 ng/ml and 0.19 ng/ml were labelled acute coronary syndromes (ACS). Skewed data was log transformed for parametric testing. Chi squared tests were used to compare proportions with results expressed as relative risks, 2 sample t tests used to compare independent group means and the Kruskall Wallis test used to compare the distribution of 3 continuous variables.
**Results:** We identified 407 subjects with elevated troponin t concentrations. Data for absolute troponin t concentrations was available for 197 subjects, median = 0.185 ng/ml (IQR: 0.07 to 1.035). 3% of subjects represented to our hospital and had a raised troponin t concentration in the following year. Glucose concentrations were recorded in 200 subjects and diabetes classified according to the ADA guidlines. 79% of subjects did not have diabetes, 15% had type 1 or 2 diabetes, 6% impaired fasting glucose and none had impaired glucose tolerance. One year all cause mortality was 35%. All cause mortality for subjects with and without diabetes was 47% and 27% respectively. There was no significant difference in troponin t concentration between those alive and dead at 1 year, median (IQR); 0.23 ng/ml (0.08 to 1.24) and 0.13 ng/ml (0.065 to 0.51). When compared with AMI, ACS conferred a significant mortality disadvantage at 1 year RR = 1.4 (95% CI = 0.97 to 2.15, Chi-squared = 4.03, df = 1, $P$ = 0.045). Diabetes or ACS did not significantly increase the frequency of troponin t elevations in the following year. The presence of diabetes or impaired fasting glucose did not affect the presenting absolute troponin t concentration or diagnosis of AMI/ACS. However, diabetes adversely affected 1 year all cause mortality RR = 1.2 (0.65 to 2.39, $P$= 0.032).
**Conclusion:** In our population of subjects with elevated troponin t concentrations, diabetes confers a significant survival disadvantage despite adherence to current UK guidelines for the management of acute coronary syndromes.

## 1182

**Prognosis and risk stratification of diabetic patients after coronary stenting: impact of silent ischemia on cardiovascular events**

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**Background and aims:** We want to evaluate the role of myocardial perfusion scintigraphy (MPS) in risk stratification and prognosis in diabetic patients (pts) after coronary artery stenting and to compare those results in

non-diabetic pts. Special interest was the impact of diabetes on clinical and scintigraphic indicators of restenosis and incidence of silent ischemia.
**Materials and methods:** We have evaluated 52 consecutive pts (29 male and 13 female, age 57+/−8) with successful myocardial revascularization with coronary stenting. 31(59%) pts were diabetics for more than 5 years. Stent location was 61% LAD, 15% LCx and 24% RCA. Pts were followed up for 9 months after the stenting when Tc-99 m sestamibi Gated SPECT MPS was performed. We have used 20-segment analysis with 5 point scoring system (0=normal; 4=no uptake). Semiquantitative analysis was done using summed stress score (SSS), summed rest score (SRS) and summed differential or reversibility score (SDS), to evaluate the extend of ischemic myocardium. Reversibility in a stent related coronary artery territory on semiquantitative assessed SPECT study was defined as restenosis indicator. Severity of CAD was estimated using angiographic Gensini score.
**Results:** Nine months after the stenting 12 pts (24%) had target vessel ischemia, which was silent in 47%. MPS find inducible ischemia in 64% LAD, 9% LCx and 27% RCA artery territories. Pts with target vessel ischemia had average SSS 6.7 vs. 4.2 and lower post stress LVEF ($p<0.05$) comparing to the pts with out inducible ischemia ($p<0.01$) The prevalence of silent ischemia was higher in diabetic pts −7/12 pts ($p<0.05$). They also had higher SSS-10.3 vs. 5.2 in non-diabetic pts ($p<0.01$) and SDS-3.7 vs. 2.5 ($p<0.05$). Average Gensini Score was 64.8 in diabetics vs. 49.7 in non-diabetic pts. There was no difference in presence of transit ischemic dilatation (TID). We have not registered any cardiac death or myocardial infarctions. 3 of 12 pts with target vessel ischemia and average SSS>6 had unstable angina and hospitalization in the follow up period. 2 of them were diabetics. No correlation was found between silent ischemia and duration of diabetes.
**Conclusion:** MPS is a valuable method for detection of ischemia and evaluation of prognosis in both diabetic and non-diabetic patients. Diabetics had more frequent silent myocardial ischemia after stenting, which predicted more cardiac events than did no ischemia. This was closely related to the extent of ischemia indicating the value of MPS for risk stratification after stenting, especially in diabetic patients as a high-risk population.

## 1183

**Postprandial myocardial perfusion defects in Type 2 diabetic patients. Effect of different insulin regimens**
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**Background and aims:** Abnormalities of the postprandial state induce endothelial dysfunction and are important contributing factors to the development of microvascular disease and macrovascular complications in diabetic patients. To test if postprandial vascular impairment is related to myocardial perfusion defects and to investigate the relationship between myocardial perfusion and metabolic changes.
**Materials and methods:** Study group included 15 type 2 diabetic patients (aged 48 ± 5 yrs), treated with diet, and without macro- or microvascular complications. Ten healthy subjects matched for age, sex and body mass index, constituted the controls. Patients were studied, in a double-blinded cross-over design, after an overnight fasting and 2 hrs after a mixed meal (650 Kcal, 50% carbohydrate, 30% fats, 20% proteins). Furthermore, the efficacy of two different insulin regimens (regular-insulin: RI, or insulin-analog: IA) was tested. Myocardial perfusion was assessed by contrast echocardiography. Blood samples were taken to measure fasting and postprandial glycemia, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and NEFA.
**Results:** In comparison to fasting conditon, in postprandial state, diabetic patients without insulin treatment showed a significant reduction in myocardial blood volume (MBV, 6.4 ± 0.8 vs 4.6 ± 0.4, $p<.001$), myocardial flow velocity (β, 0.68 ± 0.05 vs 0.40 ± 0.04, $p<.001$), and myocardial perfusion (MBVxβ, 3.2 ± 0.03 vs 0.18 ± 0.05, $p<.001$). Post-prandial myocardial perfusion defect persisted in diabetic patients treated with RI, while myocardial perfusion significantly increased in postprandial state in diabetic patients treated with IA (MBV, 6.89+0.4, $p<.001$; β, 0.79 ± 0.03, $p<.001$; MBF, 4.29 ± 0.5, $p<.001$). A negative linear correlation between postprandial MBF and glycaemia in diabetic patients was observed ($r=0.756$, $p<.001$).
**Conclusion:** Type 2 diabetic patients, treated with diet, exhibit abnormalities in myocardial perfusion in postprandial state. Myocardial perfusion defects are related to the level of glycemia. Treatment with insulin analog reverses myocardial perfusion defects.
*Supported by: Novo Nordisk*

# PS 121

# Cardiac complications: animal studies/genes/morphology

## 1184

**Adult mesenchymal stem cells derived from adipose tissue highly express telomerase and spontaneously differentiate into contractile cardiomyocytes and mature endothelial cells**
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**Background and aims:** Adipose tissues are strongly involved in various metabolic disorders such as obesity and insulin resistance syndrome. Changes in adipose mass are achievied through the highly controlled process of adipocyte differentiation. We hypothesized that the adipose tissue contains telomerase-positive multipotent stem cells, which can be addressed to differentiate into contracting myocytes and mature endothelial cells.
**Materials, methods and Results:** Murine, pig and dog adipose tissue was processed by collagenase to obtain adult mesenchymal stem cells (MSCs). After 5 days culture MSCs cells formed colonies generating cobblestone area. Immunofluorescence, flow cytometry and western analysis showed a substantial number of MSCs cells producing spontaneously higher levels of early markers for endothelial differentiation (CD34 and VEGF-R2 ) and expressing lower levels of the markers for mature endothelial cells such as CD31, von Willebrand factor, endothelial nitric oxide synthase (eNOS) and nitrite production. Immunofluorescence, immunoblotting and RT-PCR revealed expression of telomerase catalytic subunit (TERT) in MSCs at a level close to that in embryonic stem cells (ESCs). The assay for telomerase activity (TRAP) demonstrated the presence of biologically active telomerase in MSCs and ESCs.Immunofluorescence staining and flow cytometry showed co-localization of TERT with the intracellular accumulation of DiI-acLDL in a subset of MSCs cells (2.9 ± 0,2%) at smaller sizes and with dense nuclei. Unlike DiI-acLDL negative cells, in presence of endothelial growth factors such as VEGF and bFGF, DiI-acLDL positive cells showed capability to differentiate into mature, functional endothelial cells, by producing eNOS and nitrite, expressing higher levels of CD31, and efficiently repairing a linear scare performed on a confluent monolayer. Primary culture of MSCs were also evaluated for developmental plasticity towards myogenic and cardiomyogenic lineages by exposing the cells to cardiomiogenic inductive media for 15–20 days. RT-PCR showed that upon inductive conditions, the adipose MSCs expressed myocardin-A, a key transcription cofactor for myogenic cell development. Colonies with contractile myogenic cells emerged from telomerase-positive but not negative cells after 2–3 weeks in culture. Spontaneously contracting myocytes developed in a synchronized fashion with a rhythm of about 100 beats per min, and the visible myocyte contraction lasted at least for two weeks. Immunofluorescence and immunoblotting showed the positivity of the cardiac myogenic markers, α-sarcomeric actinin, myosin, actin and the Rynodine receptor in the proteins extracted from beating colonies but not from those without contractile cells.
**Conclusion:** Adipose tissue is a valuable source of telomerase-positive multipotent stem cells, capable of differentiating into cardiac myocytes and mature endothelial cells. These observations suggest the possibility to control the process of differentiation of adipose mass and to modulate the committment of the adipocyte into cardiovascular cell lineages, useful for therapeutic purpose in cardiovascular diseases.

## 1185

**Leukocyte-type 12-lipoxygenase-deficient mice show impaired insulin-regulated GLUT4 translocation in the heart**
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**Background and aims:** We have recently shown that 12(S)-hydroxyeicosatetraenoic acid plays a role in the organization of actin microfilaments in rat cardiomyocytes, and that inhibition of 12-lipoxygenase (12-LO) abrogates insulin-stimulated GLUT4 translocation in these cells. In the present study we used mice that were null for the leukocyte 12-LO (12-LOKO) to explore the implications of this enzyme under in vivo conditions.

**Materials and methods:** Mice with targeted mutation of the Alox15 gene were obtained from Jackson Laboratory. Animals were stimulated with insulin by tail vein injection and heart, skeletal muscle and blood samples were removed 20 min later. Insulin signalling and protein expression was assessed by immunoblotting. GLUT4 translocation was determined in plasma membrane fractions.
**Results:** Insulin induced a 60–70% reduction in blood glucose in both control and 12-LOKO mice. GLUT4 expression in heart and skeletal muscle was unaffected in KO mice. Expression of Akt was significantly reduced in skeletal muscle of male 12-LOKO mice, but was unaffected in females and in the heart. Insulin-regulated serine phosphorylation of Akt and GSK3alpha and -beta was unaltered in heart and skeletal muscle of 12-LOKO mice of both genders. Insulin induced a prominent translocation of GLUT4 to cardiac plasma membranes of control mice. This response was completely abrogated in 12-LOKO mice (male and female, n=7).
**Conclusion:** Our data show that the lack of leukocyte 12-LO does not lead to the development of an insulin resistant phenotype. However, the lack of GLUT4 translocation in the heart of 12-LOKO mice supports our hypothesis that eicosanoids participate in the regulation of cardiac glucose transport by contributing to the organization of cytoskeletal elements.
*Supported by: the German-Israeli Foundation for Scientific Research and Development (GIF)*

## 1186

**Acarbose treatment reduces cardiac ischemia/reperfusion injury in mice**
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**Objective:** Protective effects of the α-glucosidase inhibitor Acarbose have been reported for various diabetic complications such as diabetic nephropathy, diabetic retinopathy, and diabetic neuropathy. Moreover, in the STOP-NIDDM study even prediabetic patients had a reduction in cardiovascular events when treated with Acarbose. Therefore, we tested the effect of Acarbose after sucrose load on the cardiac ischemia/reperfusion injury in vivo.
**Methods and Results**: 7 days before ischemia/reperfusion mice were treated daily with placebo, sucrose (4g/kg bodyweight) or sucrose/Acarbose (10 mg/kg bodyweight) by gavage. Animals underwent subsequent 30 minutes of ischemia by coronary artery ligation and 24 hours of reperfusion *in vivo*. In the sucrose group ischemia/reperfusion damage was significantly increased (infarct/area at risk 38.8 ± 7.4 vs. 62.2 ± 4.8%, placebo vs. sucrose, p<0,05). This was prevented by Acarbose treatment (infarct/area at risk 30.6 ± 7.2%).
**Conclusion:** Repetitive postprandial hyperglycemia alone can increase the ischemia/reperfusion damage in non-diabetic animals. This effect can be prevented by treatment with the α-glucosidase inhibitor Acarbose.

## 1187

**Morphological peculiarities of heart and coronary vessels damage in Type 2 diabetes mellitus**
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**Background and aims:** At present great attention is paid to investigation of cardiovascular pathology and vascular complications in type 2 diabetes patents. The aim of the work was to study morphological peculiarities of heart and coronary vessels damage in type 2 diabetes patients with late vascular complications.
**Materials and methods:** We studied endomiocardial bioptates of 7 patients with type 2 diabetes mellitus (DM) and without coronary atherosclerosis obtained during coronarography and ventriculography. After special treatment the sections of different thickness (thin and ultrathin) were examined under light and electron microscopes.
**Results:** We revealed morphological peculiarities of myocardium and coronary vessels damage in type 2 DM patients without clinical signs of atherosclerosis and ischemic heart disease, in particular, pronounced fatty degeneration of cardiomiocites, accumulation of α - and β-glycogen in them, micromitochondriosis with marked polymorphism, miofibril lysis, sarcoplasmic reticulum defects, mitochondria destruction with crysts loss, apoptosis signs as well as arterioles and small and medium arteries changes, namely, pronounced edema of endotheliocytes, occurrence of fenestrated capillaries that are not normally characteristic of myocardium, and hypertrophy of unstriated muscle cells. These changes were responsible for decrease in useful heart work and disturbed coronary vessels function.
**Conclusion:** The morphological peculiarities of myocardium and coronary arteries damage can be indicative of united pathogenetic mechanisms of development of such diseases as type 2 DM, atherosclerosis, ischemic heart disease, and can be responsible for early development of failing heart in type 2 diabetes mellitus patients without coronary atherosclerosis.

## 1188

**Polymorphisms in the 5'-upstream region of the PKC beta gene and its influence on the promoter function**
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**Background and aims:** Protein kinase C (PKC), a serine/threonine kinase, is known to be activated in various tissues under hyperglycemic conditions. Notably, PKC beta, a member of the conventional PKC family, is the predominant isoform detected in vascular tissues and is supposed to be involved in the development of diabetic vascular complications. We have identified 5 single nucleotide polymorphisms in the upstream region of the PKC beta gene; C(-853)T, C(-546)G, A(-348)G, T(-287)C, and C(-238)G, and found that patients with -287CC and -238GG homozygotes, which were in linkage disequilibrium, showed significantly higher frequencies of cardiovascular disease compared to that in patients with other genotypes. There are 2 GC boxes in the upstream region; -94/-89, and -63/-58. Since -238C>G substitution makes a GC box (GGGCGG, the polymorphic site is underlined), we examined the effect of C(-238)G polymorphism on PKC beta promoter function.
**Materials and methods:** A DNA fragment of the promoter region of the PKC beta gene (−314/+183) containing 2 polymorphic sites, T(-287)C and C(-238)G, was amplified by PCR. For deletion analysis, various lengths of plasmid including −174/+183, −87/+183, and −55/+183 were generated. Each DNA fragment was introduced into the firefly luciferase expression vector (−314/-238C/Luc, −314/−238G/Luc, −174/Luc, −87/Luc, and −55/Luc, respectively). A reporter gene assay was performed using HepG2 cells. An electrophoretic mobility shift assay (EMSA) was performed using $^{32}$P-labeled 31bp fragment (-250/-220) with nucleotide C or G at position -238.
**Results:** In a reporter gene assay, overexpression of Sp1 significantly enhanced PKC beta promoter activity. The −314/−238G/Luc showed higher activity as compared to −314/−238C/Luc (1.23 ± 0.04 and 1.00 ± 0.02 AU, respectively, $P<0.001$). The luciferase activity decreased with the following order of constructs: −174/Luc > −87/Luc > and −55/Luc (1.01 ± 0.07, 0.70 ± 0.04, and 0.24 ± 0.02 AU, respectively, $P<0.001$). The EMSA demonstrated that the -238G DNA fragment had a 5-fold higher affinity for transcription factor Sp1, when compared with the -238C DNA fragment.
**Conclusion:** In addition to the 2 GC boxes (−94/−89 and −63/−58), another sequence (−242/−237) containing the C/G polymorphic site is a potential Sp1-binding site, and may be involved in regulation of PKC beta transcription. The C(−238)G polymorphism, which is linked to T(−287)C, may affect the susceptibility to diabetic vascular complications by altering PKC beta transcription and PKC beta pool in tissue.

## 1189

**Cardiovascular risk in Type 2 diabetes is associated with variation at the *PPARG* locus: a Go-DARTS study**
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**Background and aims:** The Ala allele of the Pro12Ala polymorphism in *PPARG* has been associated with reduced risk of type 2 diabetes, reduced body mass index (BMI), lower blood pressure and reduced risk of myocardial infarction.
The T allele of a silent variant, C1431T, in strong linkage disequilibrium with Ala12, has been associated with increased body mass. Furthermore, the strength of the association of Ala12 with reduced type 2 diabetes risk may be modulated by the presence of the T allele.
As the T1431 and Ala12 alleles appear to be associated with opposing traits, we hypothesized this may also be observed for the development of cardiovascular disease.
**Materials and methods:** We performed a cohort study of 2016 individuals, with type 2 diabetes and registered on DARTS (Diabetes Audit and Research in Tayside Scotland), who had been genotyped for both polymorphisms. Using DARTS informatics, all individuals were followed up from

recruitment to the end of the study, until a cardiovascular event (non-fatal myocardial infarction or revascularization) and/or death from any cause occurred.
Cox's regression was used to model the association of genotype with time to event. A dominant model for Pro12Ala and a co-dominant model for C1431T produced the best fit. The models included both genotypes, age at recruitment, sex and smoking status. An analysis was performed in the entire cohort, in individuals in with no history of previous events and in a group enrolled into the study aged less than 70 years. A subsequent multivariate analysis was performed included first recorded total cholesterol, HDL cholesterol, $\log_{10}$ triglycerides and mean arterial blood pressure (calculated as ((dbpx2)+sbp)/3) together with mean $\log_{10}$ BMI and years with diabetes. STATA version 7 was used for all analyses.
**Results:** At enrollment the mean age of the population was 64.4 years (SD 11.6) and the mean duration of diabetes was 7.9 years (SD 6.8). The mean follow up time for all events was 36.6 months (SD 15.0). Ala12 was associated with a significantly reduced risk and T1431 with a significantly higher risk of non-fatal cardiovascular events (Table). The strength of these associations was greater in the younger cohort with a first ever event. T1431 was also associated with an increased all cause mortality. With inclusion of conventional risk factors in the model the observed associations of genotype with outcome were modestly attenuated although remained significant. (eg. First non-fatal event <70yrs - Ala12, HR 0.22 (CI 0.08–0.72); T1431, HR 8.3 (CI 1.59–43.47))

Ala12 is associated with a reduced cardiovascular risk and T1431 with increased risk.

| | Ala12 (dominant)T1431 (co-dominant) | | | | | | |
|---|---|---|---|---|---|---|---|
| | No. events/ No. at risk | HR | CI | P | HR | CI | P |
| **All non-fatal events** | 91/2016 | 0.54 | 0.27–1.08 | 0.08 | 2.34 | 0.77–7.11 | 0.13 |
| **All non-fatal events <70yrs** | 59/1349 | 0.43 | 0.18–0.99 | 0.05 | 4.75 | 1.24–18.25 | 0.02 |
| **First non-fatal event <70yrs** | 35/1176 | 0.21 | 0.06–0.69 | 0.01 | 9.90 | 1.90–51.29 | 0.007 |
| **All events inc. death < 70yrs** | 184/1349 | 0.68 | 0.43–1.1 | 0.1 | 2.55 | 1.13–5.75 | 0.024 |
| **Death <70yrs** | 133/1349 | 0.82 | 0.49–1.39 | 0.47 | 2.61 | 1.02–6.65 | 0.045 |

HR=Hazard Ratio; CI=Confidence interval. All data corrected for age sex and smoking status

**Conclusion:** This study confirms the association of Ala12 with reduced risk of myocardial infarction in a type 2 diabetic population, and demonstrates T1431 is associated with an increased risk. These associations appear largely independent of intermediate traits conventionally associated with cardiovascular disease.
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## 1190

**Peroxisome proliferator-activated receptor-gamma2 Pro12Ala polymorphism is associated with reduced risk for ischemic stroke with Type 2 diabetes**
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**Background and aims:** Peroxisome proliferator-activated receptor (PPAR)-gamma2, 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 ischemic stroke in type 2 diabetes. Recently, the Ala allele of the common Pro12Ala polymorphism in the isoform PPAR-gamma2 has been shown to be associated with reduced risk for type 2 diabetes and its complications. We have studied the association of this polymorphism with ischemic stroke in a Korean non-diabetic and type 2 diabetic populations.
**Materials and methods:** Three hundred and two ischemic stroke patients (131 with type 2 diabetes), 284 healthy matched control subjects and 141 type 2 diabetic patients without ischemic stroke (diabetes duration ≥ 10 years) were evaluated. The PPAR-gamma2 Pro12Ala polymorphism was analyzed by PCR-RFLP.
**Results:** PPAR-gamma2 Pro/Ala genotype and Ala allele frequency were lower in ischemic stroke patients than those observed in the control group (4.0 vs. 9.2%, OR=0.41, P=0.0109; and 2.0 vs. 4.6 %, OR=0.42, P=0.0123; respectively). Genotypic analysis revealed that ischemic stroke patients with type 2 diabetes displayed a great lower prevalence of the Pro/Ala genotype (2.3%) than controls (9.2%)(OR=0.23, P=0.0116). Especially, Pro/Ala genotype and Ala allele frequency of type 2 diabetes patients with ischemic stroke were lower than type 2 diabetes patients without ischemic stroke (2.3 vs. 8.5%, OR=0.25, P=0.0321; 1.1 vs. 4.3%, OR=0.26, P=0.0345).
**Conclusion:** These results suggest that the Ala allele of PPAR-gamma2 Pro12Ala polymorphism may be associated with reduced risk for ischemic stroke with type 2 diabetes and it could be an useful predictive marker for ischemic stroke in type 2 diabetes.
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## 1191

**p66shc gene is expressed in human peripheral blood mononuclear cells: the effect of diabetes**
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**Background and aims:** Oxidative stress plays an important role in cardiovascular dysfunction and atherogenesis. This is of particular interest in diabetes, a condition characterized by oxidative stress and increased prevalence of cardiovascular disease. p66shc role in oxidative stress-related response has been demonstrated by resistance to and reduction of oxidative stress and prolonged lifespan of p66shc-/- mice. Our study assesses p66shc gene expression in mononuclear cells (PBM) from type II diabetic patients.
**Materials and methods:** p66shc mRNA level was assessed in PBM from type 2 diabetes patients and healthy subjects using RT-PCR with two sets of primers mapping for different p66shc regions.
**Results:** p66shc is expressed both in monocytes and lymphocytes. In addition, p66shc mRNA level was significantly higher in type 2 diabetic patients compared to controls:0.38 ±;0.07 d.u. vs 0.13 ±;0.08, p<0.0001.
**Conclusion:** In type 2 diabetic patients p66shc, which plays a critical role in induction of oxidative stress related responses, is not only expressed in peripheral blood mononuclear cells but is also upregulated. To our knowledge, this is the first demonstration of increased p66shc gene expression in type 2 diabetic patients. The demonstration that p66shc is expressed in PBM makes these easy accessible cells a useful tool to investigate processes involved in diabetic complications such as cardiovascular remodeling and atherogenesis.

## 1192

**K121Q polymorphism of the plasma cell membrane glycoprotein is associated with early atherosclerosis in patients with Type 2 diabetes**
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**Background and aims:** The K121Q polymorphism of the plasma cell membrane glycoprotein (PC-1) has been shown to be associated with insulin resistance. No data are available on its association with intima-media thickness (IMT), a generally accepted good marker of early atherosclerosis. Therefore, the aim of our study was to investigate the relationship between the K121Q polymorphism of the PC-1 gene and carotid IMT.
**Materials and methods:** Altogether 617 subjects (282 men, 335 women) at high-risk for type 2 diabetes from the Risk factors in IGT for Atherosclerosis and Diabetes (RIAD) study, were included in this study. Carotid IMT was measured by B-mode ultrasound. Plasma glucose, lipids, inflammatory, fibrinolytic and coagulation parameters were examined by conventional methods. Insulin was determined by highly specific enzyme immunoassay and albuminuria by nephelometry. Genotyping of the K121Q polymorphism of the PC-1 gene was performed by the polymerase chain reaction amplification. The products were visualized with the single strand conformational polymorphism method.
**Results:** A total of 90 from the RIAD participants had newly detected type 2 diabetes. The genotype frequencies among non-diabetic subjects were as follows: 386 of them had the common K121K genotype, 136 had the K121Q genotype and 5 subjects had the Q121Q genotype. Among diabetic patients the K121K genotype was observed in 70, the K121Q genotype in 18 and the Q121Q genotype in 2 subjects. Diabetic 121Q allele carriers had higher level of albuminuria compared to diabetic subjects with the K121K genotype (24.11 vs. 17.54 mg/l; p=0.020). There was no difference in IMT among the genotype groups in non-diabetic subjects. Carotid IMT was significantly increased among diabetic subjects who were carriers of the Q allele compared to the K121K genotype in diabetic subjects (1.05 vs. 0.92 mm, p=0.014) and this result remained significant after adjustment for albuminuria. In multivariate analysis including various cardiovascular risk

factors the K121Q polymorphism of the PC-1 gene was found to be an independent determinant of early atherosclerosis among diabetic patients.
**Conclusion:** The Q allele of the K121Q polymorphism of the PC-1 gene is associated with early atherosclerosis in subjects with type 2 diabetes.

## 1193

**A component of the diabetic angiopathy – hyaluronic acid – promotes the development of atherosclerosis**
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**Background and aims:** The very high and equal frequency of cardio-vascular disease (CVD) among men and women with diabetes is still mysterious. It is known that the vessel wall thickness is increased in young patients who are normolipaemic and normotensive. Furthermore, histochemical and biochemical investigations show that atheromatosis free arterial wall segments among other components of the extra-cellular matrix contain increased levels of HA in the tunica media (TM). These changes may facilitate the development of atherosclerosis and thereby contribute to increased frequency of CVD in diabetic patients. Our aim is to explore whether increased HA content in TM renders the vessel wall more susceptible to atherogenic insults.
**Materials and methods:** We have established transgenic mice with overexpression of human hyaluronan synthase2 (hHAS-2) gene in the smooth muscle cells (SMC) of the TM. The hHAS-2 transgenic mice were bred with ApoE-deficient mice to obtain hHAS-2$^+$ApoE–/– mice and hHAS-2$^-$ApoE–/– mice for our investigations. After 4 to 5 months on normal diet, mice were sacrificed and three sections of the aortic root were cut with standardized intervals (0–80µm–160µm) and stained with elastin. The atherosclerotic lesion area in each section was determined in a blinded fashion by lightmicroscopy and Analysis software. The extent of cholesterol deposits within the aortic arch and the thoracic aorta were visualized by en face staining with Oil Red O. The percentage of the arch covered by lesions was calculated by means of Analysis software including multiple images alignment. The area with positive staining in the thoracic aorta was calculated by using a frame (175,349.1 µm$^2$/144 cross) that was moved with standardized intervals (1 frame) from side to side along the aorta. Quantitative real-time polymerase chain reaction was used to study gene expression of collagen receptor discoidin domain receptor1 (DDR1) in 4 months old hHAS-2+ and hHAS-2- mice.
**Results:** In the aortic root we found the extent of the atherosclerotic lesions were higher in hHAS-2$^+$ApoE–/– mice (n=15) versus hHAS-2$^-$ApoE–/– mice (n=9) when the two most distal sections were evaluated (both $p<0.05$). In the en face analysis of the aortic arch we found a significant increase in the area (%) of lipid deposits in hHAS-2$^+$ApoE–/– mice (n=17) compared with hHAS-2$^-$ApoE–/– mice (n=10) ($p<0.001$). This difference was also present in the descending aorta ($p<0.05$). Preliminary results show that the expression of DDR1 from aorta was lower in the hHAS2+ mice (n=4) compared with hHAS-2– mice (n=6) (p=0.04).
**Conclusion:** Increased amounts of HA in tunica media accelerates atherosclerosis in ApoE–/– mice. Thus, increased HA levels in the tunica media, a part of the diabetic angiopathy, may play a critical role in the progression of atherosclerosis in patients with diabetes. The lower DDR1 expression in the aorta of hHAS-2 transgenic mice may indicate that the SMC loose signals from the microenvironment, thereby changing SMC phenotype.

# PS 122
# QTc interval/autonomic neuropathy and CHF

## 1194

**Diabetes mellitus attenuates the repolarization reserve in canine heart**
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**Background and aims:** In diabetes mellitus several cardiac electrophysiological parameters are known to be affected. In rodent experimental diabetes models changes in cardiac electrophysiological parameters were reported, but no such data are available in other mammalian species including dog. Therefore, we have measured and compared the QTc intervals, the action potentials and the magnitude of several transmembrane currents in right ventricular papillary muscles and in myocytes isolated from control and alloxan induced diabetic dog hearts.
**Materials and methods:** Diabetes was induced in mongrel dogs (n=8) by iv. administration of 510 µg/kg alloxan, and the study was performed 5–6 weeks after. Non-diabetic dogs (n=8) were used as controls. Surface ECG, conventional microelectrode technique and single cell patch clamp method were used to characterize the electrophysiologocal changes. Expression of ion channel proteins was assessed by Western blotting.
**Results:** The QTc values before and after alloxan (diabetes) were not statistically significant (191.0 ± 9.3 ms *vs* 196.0 ± 14.7 ms, respectively). The action potential duration was somewhat longer in papillary muscles obtained in diabetic dogs [190.3 ± 3.5 ms in control (n=26) *vs* 204.5 ± 4.8 ms in diabetes (n=25); $p<0.05$ at stimulation cycle length of 500 ms]. The amplitude of IKs and Ito measured in cardiomyocytes from diabetic dogs were significantly decreased when compared with that in the control dogs [IKs, measured at 50 mV, 1.40 ± 0.13 pA/pF in control (n=31) *vs* 0.97 ± 0.10 pA/pF in diabetic dog (n=32); $p<0.05$; Ito, measured at 50 mV, 17.39 ± 1.92 pA/pF in control (n=32) *vs* 9.54 ± 1.11 pA/pF in diabetic dog (n=35); $p<0.05$]. The amplitudes of ICa, IK1 and IKr did not change. In agreement with this finding using the Western blot technique it was found that the expression level of Kv4.3 and minK protein was also decreased.
**Conclusion:** The alloxan induced diabetes does not markedly lengthen the repolarization but attenuates the safety margin of the repolarization („repolarization reserve") by decreasing IKs and Ito, and as such, it may increase the proarrhythmic risk.
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## 1195

**Effects of coffee intake on aortic pressure waveform in subjects with Type 2 diabetes**
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**Background and Aims:** Coffee is the most abundantly consumed stimulant worldwide. In non-diabetic subjects coffee intake acutely increases the pulse wave reflection (Ag) of the aorta, which is a measure of systemic arterial stiffness. Increased Ag is an independent predictor of cardiovascular mortality. The effect, however, of coffee consumption on Ag in patients with diabetes is not known. In this study we examined the effect of coffee intake on Ag in patients with type 2 diabetes (T2DM).
**Materials and Methods:** A total of 15 patients (men: n=8; women: n=7; mean age 56.5 ± 8.7 years; $HBA_{1c}$: 7.4 ± 1.2%) with T2DM and acceptable metabolic control were examined. Participants attended the metabolic unit of our department early in the morning after fasting for 12–14 hours twice; in one visit they received a standard instant coffee beverage (240 ml) containing 80 mg of caffeine and in a second visit equal amount of water. Coffee and water were given in random order. Radial artery and aortic blood pressure were measured using the applanation tonometry method at

baseline and 1, 2, and 3 hours after coffee or water consumption. Pulse wave analysis was used to calculate Ag of the aorta.
**Results:** Ag was not different at the examined times after water or coffee consumption: baseline: 17.5 ± 2.9 vs 17.3 ± 2.5 mmHg (P=0.94); 1st hour: 21.5 ± 5.1 vs 20.1 ± 1.9 mmHg (P=0.74); 2nd hour: 19.5 ± 3.2 vs 21.0 ± 2.6 mmHg (P=0.58); 3rd hour: 17.7 ± 3.4 vs 17.3 ± 3.2 mmHg (P=0.88). No significant changes in Ag were observed during the study in comparison with the baseline values after either coffee or water intake (P>0.05, ANOVA for repeated measurements). In addition, radial and central aortic systolic and diastolic blood pressure also did not differ significantly between the two phases of the study.
**Conclusions:** Acute moderate coffee intake does not affect the pulse wave reflection of the aorta in patients with T2DM. In addition, coffee does not affect either the radial or the aortic pressure in these patients. These findings suggest that moderate coffee consumption does not have any harmful effect on systemic arterial stiffness in patients with type 2 diabetes.

## 1196

**Effects of coffee intake on QT interval in subjects with Type 2 diabetes**
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**Background and Aims:** Caffeine has sympathoexcitatory effects and recent data from the general population has shown that it prolongs the corrected QT interval (QTc) of the resting electrocardiogram (ECG). Prolongation of the QT interval predisposes to ventricular arrhythmias. The effect, however, of coffee intake on QT interval duration in patients with diabetes is not known. In this study we examined the effect of coffee consumption on QT interval and its dispersion (QTd) in patients with type 2 diabetes (T2DM).
**Materials and Methods:** A total of 15 patients (men: n=8; women: n=7; mean age 56.5 ± 8.7 years; $HBA_{1c}$: 7.4 ± 1.2%) with T2DM examined. Participants attended the metabolic unit of our department early in the morning after fasting for 10–12 hours twice; in one visit they received a standard instant coffee beverage (240 ml) containing 80 mg of caffeine and in a second visit equal amount of water. Coffee and water were given in random order. Resting ECG recordings were performed at baseline and 1, 2, and 3 hours after the coffee or the water consumption. The paper recordings were then scanned to an image at high resolution, edited, and converted to a digital ECG recording, which was analyzed interactively using an ECG analysis program. QTd was calculated as the difference between the maximum and the minimum QT intervals in any of the 12 leads. QTc interval was calculated using the Bazett's formula.
**Results:** Coffee, in comparison with water intake, resulted in a significant prolongation of the QT interval duration at the 1st and 2nd hour after the experiment [1st hour: 414.9 ± 58.1 vs 368.6 ± 38.9 msec (P<0.0001); 2nd hour: 397.5 ± 55.8 vs 360.4 ± 69.9 msec (P=0.04), respectively]. No significant differences were found at baseline or the 3rd hour between the two phases of the study. The same was valid for QT max [1st hour: 456.1 ± 59.8 vs 412.5 ± 42.5 (P=0.007), and 2nd hour: 436.7 ± 73.4 vs 394.9 ± 76.2 msec (P=0.03), respectively]. QTd did not differ significantly at the studied times between the two phases of the study. In addition, corrected QT interval (QTc) was more prolonged 1 and 2 hours of the study after coffee in comparison with water consumption [425.9 ± 32.4 vs 386.4 ± 27.6 msec, (P<0.0001) and 407.9 ± 39.9 vs 374.4 ± 45.6 msec (P=0.006), respectively].
**Conclusions:** Acute moderate coffee intake increases QT interval in patients with T2DM. This effect suggests that coffee prolongs the ventricular repolarization time and may predispose to ventricular arrhythmias in patients with T2DM.

## 1197

**Effect of acute hyperhomocysteinaemia on QT interval in patients with Type 2 diabetes**
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**Background and aims:** An increased QT interval duration represents prolongation of the depolarization and repolarization time of the cardiac ventricles and it is an independent predictor of high cardiovascular mortality in both diabetic and non-diabetic subjects. We have previously shown that acute, methionine (M)-induced hyperhomocysteinemia (h-Hcy) increases cardiac sympathetic nervous system (SNS) activity in subjects with type 2 diabetes mellitus (T2DM). Previous studies have shown that an increased cardiac SNS activity affects QT interval duration. However, the potential effect of h-Hcy on QT interval has not been examined so far.
**Material and methods:** A total of 13 subjects with T2DM [7 males, 6 females; mean age: 57.3 ± 8.7 years, mean duration range of diabetes: 9.9 (1–20) years], non-smokers and without macrovascular complications were studied. Subjects were examined on two separate days with an interval of about 1 week in between. In the first visit, 250 cc of water were given while in the second visit 0.1 g/Kg of body weight of M diluted in the same amount of water. Plasma homocysteine (Hcy) levels were measured at baseline and 3 hours after the M or the water load. QT, QT interval corrected for heart rate (QTc) and their dispersions (QTd and QTcd) were also measured at the same time.
**Results:** Plasma Hcy at baseline was 12.5 ± 8.1 µmol/l and at 3 hours after M 34.9 ± 9.2 µmol/l (P<0.0001). Plasma Hcy levels did not change after the water load (12.6 ± 7.7 and 12.8 ± 7.8 µmol/l, P=0.87). The duration of QT increased from 315.0 ± 81.7 msec at baseline to 339.3 ± 96.3 msec (P=0.02) at 3 hours after M, while no significant change was observed after the water load (322.5 ± 73.3 msec at baseline; 321.3 ± 81.7 msec at 3 hours, P=0.80). Similarly, QTc increased from 353.5 ± 50.0 msec to 380.9 ± 54.4 msec (P<0.0001) after M but no after water (P=0.42). QTd and QTcd did not change significantly after either water or M intake.
**Conclusion:** An acute increase of plasma Hcy results in a significant prolongation of QT interval duration in subjects with T2DM. This novel finding suggests that h-Hcy causes prolongation of the electrical activity of the cardiac ventricles and may predispose in ventricular arrhythmias.

## 1198

**P-wave dispersion and atrial fibrillation in Type 2 diabetes mellitus**
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**Background and aims:** Heart rate disturbances, including atrial fibrillation (AF) are often observed in patients (pts) with type 2 diabetes (T2DM). It has been shown, that P-wave dispersion (Pd) may be used for predicting pts with paroxysmal atrial fibrillation from healthy subjects. The aim of this study was to evaluate the relation between P-wave dispersion and AF in pts with T2DM during sinus rhythm.
**Materials and methods:** Participants were 143 pts with T2DM. All pts were in sinus rhythm. In all pts 12-lead surface ECG, 24- hour Holter ECG and echocardiography were performed. The maximum P-wave duration (P-max), the minimum P-wave duration (P-min) and P-wave dispersion (Pd=Pmax-Pmin) were calculated on the surface ECG. The rhythm disturbances were studied using the Holter ECG. Left ventricular ejection fraction (LVEF) and left atrial diameter (LAD) were measured using echocardiography. According to the Holter ECG data pts were divided into 3 groups. (Gr.). Gr. 1 – 49 pts (29 men/20 women, mean age 59 ± 11 yrs) with episodes of AF on Holter ECG; Gr. 2– 46 pts (25 men/24 women, mean age 58 ± 13 yrs) with frequent supraventricular extrasystoles (SVE>1000/24 h) and runs of supraventricular tachycardia (SVT) on Holter ECG and Gr. 3–48 pts (26 men/22 women, mean age 60 ± 12yrs.) without supraventricular arrhythmia or with only infrequent isolated SVE.
**Results:** There was no significant difference in age and sex between the groups. P dispersion was significantly higher in Gr. 1 and Gr. 2 than in Gr. 3. (52 ± 9 vs 35 ± 10 ms, p<0.001; 48 ± 13 vs 35 ± 10 ms p<0.001, respectively). The highest Pd was found in Gr. 1, but it did not differ significantly from Pd in Gr. 2. There was not significant difference in P-max between the groups (124 ± 13; 122 ± 12; 119 ± 14 ms, respectively), whereas P-min was significantly lower in Gr. 1, than in Gr. 3 (72 ± 11 vs 81 ± 8 ms, p<0.001). P-min was also lower in Gr. 2 than in Gr. 3, but difference was not significant (78 ± 9 vs 81 ± 8 ms). There were no significant differences in LAD between the three groups (39.6 ± 4.2; 40.3 ± 3.8; 38.5 ± 3.6 mm, respectively). Whereas, LVEF was found significantly lower in Gr. 1 and Gr. 2, than in Gr.3. (55 ± 3 vs 62 ± 5%, p<0.001; 56 ± 4 vs 62 ± 5%, p<0.001, respectively). Thus, AF and SVT in pts with T2DM are associated with increased P-wave dispersion on surface ECG.
**Conclusion:** Increased P-wave dispersion may be a useful, simple, non-invasive marker for assessment of development of atrial fibrillation and supreventricular tachycardia in pts with T2DM during sinus rhythm.

## 1199

**The effect of the anticholineesterase Edrophonium on heart rate and left ventricular diastolic function in Type 1 diabetic patients with cardiovascular autonomic neuropathy**

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**Background and aims:** To test the hypothesis that unopposed sympathetic stimulation in diabetic patients with early cardiovascular neuropathy may cause left ventricular stiffening and hypertrophy, we have evaluated the effect of pharmacologically induced parasympathetic stimulation on myocardial function in type 1 diabetic patients with and without parasympathetic cardiovascular neuropathy.
**Materials and methods:** Ten type 1 diabetic patients with autonomic neuropathy, defined by cardiovascular tests (AN+) and ten age- and sex matched patients without neuropathy (AN-) as well as ten healthy subjects (C) participated in the study. Urinary albumin excretion rate was not significantly different in the diabetic groups. Ten mg Edrophonium Hydrochloride was infused intravenously, while a continous 12-lead ECG was recorded and left ventricular diastolic function was assessed by Doppler echography.
**Results:** Baseline heart rates were AN+ 77 +/- 2 beats/min (mean +/- S.E.M.), AN- 68 +/- 2, C 63 +/- 1. The decrease in heart rate on Edrophonium infusion was AN+ 0.2 +/- 0.6, AN- 7.7 +/- 1.1 and C 8.4 +/- 0.9 (AN+ vs. AN-; $p<0.03$, AN+ vs. C and AN- vs C; $p<0.0001$). Before Edrophonium infusion there were significantly higher A-waves in AN+ than in AN- and C ($p<0.025$). Also, the deceleration time was different, AN+ having significantly lower values ($p<0.025$). E/A ratio before Edrophonium infusion was not significantly different between the groups; after Edrophonium, E/A ratio was significantly lower in AN+ (0.94 +/- 0.06) compared with AN- (1.36 +/- 0.13) and C (1.39 +/- 0.10) ($p<0.01$), mainly due to a significant increase in E-wave in AN- and C, which did not occur in AN+.
**Conclusion:** Type 1 diabetic patients with predominantly parasympathertic cardiovascular autonomic neuropathy do not have the ability to decrease heart rate and increase myocardial distensibility upon parasympathetic stimulation, probably due to neuropathic destruction of parasympathetic nerve terminals. This finding is in accordance with the hypothesis that unopposed sympathetic stimulation may contribute to impaired myocardial function in cardiovascular diabetic autonomic neuropathy.

## 1200

**Spectrum of haemodynamic autonomic dysfunction in older patients with Type 2 diabetes mellitus**

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**Background and aims:** Cardiovascular autonomic dysfunction (CVAD) is an important diabetic complication and indicator of poor prognosis, affecting 7–40% of patients. Although many studies have documented orthostatic hypotension (OH) and a few postprandial hypotension (PPH) in type 2 diabetes (T2D), other abnormal haemodynamic responses during every day life have not been investigated. The mechanism(s) and role of different risk factors in CVAD are also not well understood. The aims of the study were to evaluate the types of CVAD, their prevalence and potential determinants in a single cohort of older (50–76 years) patients with T2D.
**Materials and methods:** We studied 123 patients (mean age 61.3 ± 7.4(SD) years; 73 men, 50 women) with T2D. 39 subjects were managed by diet alone, 74 were receiving oral hypoglycaemic drugs and 14 have been treated with insulin. The mean duration of DM was 6.6 ± 6.2 years. Body mass index (mean 31.0 ± 7.1 kg/m$^2$) was >25 kg/m$^2$ in 87% and > 30 kg/m$^2$ in 47% of patients. Changes in blood pressure (BP) and heart rate (HR) were measured in the following conditions: (1) from lying to standing (for 3 min), (2) from sitting to standing, (3) after a 6 min walk, (4) one hour after mixed meal (450 kcal) sitting, than (5) standing and (6) after a walk. In blood samples taken after an overnight fast concentrations of glucose, glycahaeglobin (HbA1 c), cholesterol, triglyceride, HDL cholesterol, creatinine and urea nitrogen were measured.Urine albumin and creatinine excretion were also measured. OH and PPH were defined as a decrease of systolic/diastolic BP by more than 20/10 mmHg on standing within 3 min or 1 hour after meals, respectively. Orthostatic hypertension (OHT) and postprandial hypertension (PPHT) were defined as an icrease in systolic/diastolic BP more than 10% from baseline.
**Results:** At least one abnormal BP response was present in 43 (35%) of subjects. OH was found in 8 (6.7%) and OHT in 18 (14.6%). Chronotropic incompetence (rise in HR less than 10% on standing) was observed in 34 (27.6%) subjects. PPH was diagnosed in 12 (9.7%) subjects and this increased to 13 (10.6%) after standing and decreased to 5 (4.1%) with walking. OH and PPH occurred in 3 (2.4%), OHT and PPHT in 1 (0.8%), OHT and PPH in 3 (2.4%) and OH and PPHT in 1 (0.8%) subject. There were no significant differences in age, gender, age of DM onset, disease duration, BMI, use of medications, fasting glucose, HbA1 c, lipids ,or pulse pressure in subjects with or without CVAD. However, when considered by types of CVAD, patients with hypotensive responses have significantly higher baseline BP(154 ± 21/86 ± 13 vs 138 ± 16/80 ± 11). OH was independently and significantly assosiated with microalbuminuria ($p<0.001$) and albumin-to-creatinine ratio ($p<0.001$). Moreover, orthostatic decline in BP also significantly correlated with these determinants ($p<0.001$).
**Conclusion:** CVAD occurs frequently in older T2D patients even with preclinical autonomic neuropathy and has a wide spectrum of presentations. Simple measurements such as BP and HR recorded on active change in position from lying to standing and after meals may be helpful in detecting CVAD in T2D patients. Different types of CVAD may have different pathophysiology and, therefore, different prognostic value in these patients.

## 1201

**Diabetics with heart failure have more prominent autonomic dysfunction than non diabetic heart failure patients**

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**Background and aims:** Heart failure (HF) nowadays becomes more frequently recognized as advanced cardiovascular complication of diabetes mellitus type 2 (DM2). Little is known about influence of DM2 on autonomic dysfunction in heart failure patients. The **aim** of this study was to investigate weather DM2 aggravate heart rate variability (HRV) in heart failure patients
**Materials and methods:** HeRaVa pilot study, designed to analyze short term HRV in heart failure. In this section we compared HRV profile of 10 heart failure diabetics and 53 heart failure patients without DM2 similar in age, ejection fraction and NYHA class. Frequency domain (VLF, LF, HF, LF/HF LF (nu) and HF (nu)) parameters were determined by commercial equipment (Schiller AT-60). HRV was measure in basal condition (1024 QRS), during deep breathing test (128 QRS during normal and 128 QRS during deep breathing with 0.1Hz rate) and during active tilt test (256 QRS in supine position and 256 QRS after one minute in standing position). Results were statistically tested with student t test after ln transformation.
**Results:** In basal condition following HRV parameters was markedly reduced in heart failure diabetics compared to heart failure patients without DM2: Tot Power (747.77 ± 630.03 vs. 579.82 ± 748.49 ms$^2$, p=0.050), VLF (171.06 ± 152.39 vs. 94.11 ± 90.44 ms$^2$, p=0,022), LF (178.34 ± 214.83 vs. 62.22 ± 66.47 ms$^2$, p=0.007) and LF (29.31 ± 16.81 vs. 15.35 ± 9.14 nu, p = 0,003). Diabetics with heart failure have lower HRV during spontaneous breathing (SB). During deep breathing (DB) diabetics could not achieve such increment in HRV like patients without DM2. Non diabetics during deep breathing significantly increased following HRV parameters compared with spontaneous breathing: LF (28,93 ± 37,29 vs. 102,77 ± 96,72 ms2, p=0,000), HF (10,33 ± 14,20 vs. 40,14 ± 64,35 ms2, p=0,000), Tot Power (77,06 ± 74,89 vs. 174,13 ± 145,51 ms2, p = 0,000), LF (64,92 ± 27,15 vs. 76,32 ± 20,44 nu, p=0,000). The power in HF range in this group during deep breathing was reduced compared with spontaneous breathing (27,19 ± 18,48 vs. 21,76 ± 18,53 nu, p=0,049). Type 2 diabetics significantly increased following HRV parameters: LF (11,56 ± 14,10 vs. 65,44 ± 96,37, ms2, p = 0,024), Tot Power (51,87 ± 68,90 vs. 126,24 ± 153,56 ms2, p=0,047), LF (48,32 ± 27,57 vs. 68,35 ± 26,81 nu, p=0,048)
**Conclusion:** Diabetes mellitus type 2 aggravates autonomic dysfunction in heart failure patients. This can be easily acquired by spectral analyses of short term HRV in basal condition. Deep breathing pronounced differences in HRV between these groups. Although both diabetics and non diabetics increased HRV during deep breathing, the number of parameters significantly changed and value of increments are significantly lower in diabetics. This can suggest more profound autonomic disturbances (lower possibility to increase baroreflex sensitivity).

# PS 123

# Cardiac complications: risk engines/effects of metabolic control

## 1202

**Cardiovascular risk in Type 2 diabetics. Framingham equation scores lower than UKPDS**

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**Background and aims:** Identify high risk diabetic patients, is important to select those who may benefit from more intensive interventions. This study aims to compare cardiovascular risk in type 2 diabetics (DM2) accessed by Framingham and UKPDS equations.
**Materials and methods:** Consecutive ambulatory patients with type 2 diabetes were selected from 1996 to 2003, from the clinical database. UKPDS risk was calculated with the Risk Engine available at UKPDS Web Site. Fifty two items were recorded in relation with diabetes history, late manifestations, concomitant diseases, laboratorial parameters and ongoing therapy. Data were analyzed with the software SPSS, and presented by average and standard deviation (STD).
**Results:** Three hundred and twenty patients were selected, 153 males and 167 females, 316 caucasians. Prevalence of smokers and atrial fibrillation was low in this population. Cardiovascular risk events at ten years are presented at the following table.
**Conclusion:** Many published data show that Framingham equation underestimates the cardiovascular risk when applied to a diabetic population. The UKPDS risk engine model is diabetes-specific and incorporates glycemia, systolic blood pressure and lipid levels as risk factors, in addition to age, sex, ethnic group, smoking status and time since diagnosis of diabetes. In this population of type 2 diabetic patients, the average value calculated by Framingham equation is clearly below the expected. Older patients with long duration of diabetes presented the most important differences at risck socres. Although both methods identify at-risk individuals, with UKPDS equation a higher proportion of individuals candidates for multifactorial intervention are identified.

Population Main Characteristics

| | Age (years) | Diabetes Duration (years) | CHD Risk Framingham | CHD Risk UKPDS | Hb $A1_c$ (%) | Systolic BP (mm Hg) | Cholesterol (mmol/L) | HDL (mmol/L) |
|---|---|---|---|---|---|---|---|---|
| Average | 60.7 | 12.9 | 19.3 | 50.0 | 8.3 | 147.5 | 5.8 | 1.2 |
| STD | 11.4 | 11.4 | 10.6 | 32.5 | 2.2 | 20.4 | 1.4 | 0.3 |

## 1203

**The UKPDS risk engine predicts higher 10-year absolute coronary artery disease risk than the Framingham risk function in a district general diabetes clinic population**

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**Background and aims:** The use of the Framingham risk function (FRF) for 10-year absolute coronary artery disease risk (CHD) prediction in a diabetes population has been questioned given that the proportion of diabetes subjects in the Framingham Heart Study was only 6%. Despite this concern, it currently remains the gold standard for risk estimation in UK guidelines. We have compared risk scores as predicted by the UKPDS Risk Engine (UKRE) and the FRF in a secondary referral diabetes clinic.
**Materials and methods:** Diabetes and cardiovascular parameters were analysed on 1000 consecutive patients. Of these, 400 were patients with type 2 diabetes mellitus without established atherosclerotic disease. Data was collected as for the Alphabet Strategy Audit template. The Alphabet Strategy is diabetes care based around the mnemonic: A – advice; B – blood pressure control; C – cholesterol profile; D – diabetes control; E – eye examination; F – foot examination; G – guardian drugs (aspirin, ACE inhibitors, and lipid lowering therapy). 10-year absolute CHD risk was calculated using the Cardiac Risk Assessor (based on Framingham equations) and the UKPDS Risk Engine. Statistical analysis was performed using Microsoft Excel.
**Results:** Mean CHD risk estimates using the UKRE and FRF were 17.7 ± 12.2% and 13.5 ± 7.2% respectively. 58% were males. Mean age was 58 years. When data was categorised into treatment ranges of ≤ 15% and >15% as specified in European guidelines, the FRF classified 64% (256) of patients below the 15% treatment cut-off. Of these patients, the UKRE would classify 55 (21.5%) in the >15% range. Conversely, in those whom the FRF classified as >15% (36% of patients), the UKRE would not treat 11 (7.6%).
**Conclusion:** This study suggests that the UKRE estimates CHD risk higher than with the FHF method. Our results support the argument that the FRF underestimates relative to the UKRE in diabetes patients.

## 1204

**Cardiovascular risk assessed through the UKPDS risk engine in Type 2 diabetes persons**

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**Background:** It is unanimously recognized that Type 2 diabetes is associated with a high risk for cardiovascular disease, due to multiple risk factors. Complex and intensive intervention is beneficial. To optimize the clinical management, a global cardiovascular risk assessment should be performed and specific interventions should be implemented.
**Aim:** The aim of this analysis was to evaluate the risk for coronary heart disease (CHD) and stroke in Type 2 diabetes persons.
**Studied group and Methods:** A number of 400 Type 2 diabetes persons have been randomly included. Demographic, clinical and biochemical data have been recorded. Based on The UKPDS Risk Engine, the risk for overall and fatal coronary heart disease and overall and fatal stroke, over a period of 10 years, were assessed.
**Results:** Mean age was 60.2 (± 9.4) years; mean duration of diabetes was 7 (± 5.4) years. According to anthropometrical parameters, 45.7 % were overweight and 43.2 % obese; 78.7 % of women and 44 % of men were with large waist (ATP III). Regardless therapy, 11 % of the patients were on diet, 63 % on oral therapy (monotherapy 50.6 % and combined oral therapy 49.4 %) and 26.11 % on insulin. The global and fatal coronary heart disease risk and the global and fatal risk for stroke are presented in Table 1.

Table 1 Risk for CHD and stroke

| Risk | < 10 % | 10 – 19 % | 20 – 39 % | ≥ 40 % | Mean (± SD) |
|---|---|---|---|---|---|
| - for CHD (%) | 21.7 | 35.5 | 35.8 | 6.8 | 20.2 (12.5) |
| - for fatal CHD (%) | 48.2 | 31.5 | 17.4 | 2.9 | 13.5 (10.6) |
| - for stroke (%) | 65.5 | 23.2 | 9 | 2.2 | 9.8 (9.6) |

The mean risk for fatal stroke was 1.6 (± 1.7).
There is no correlation between CHD and therapeutic structure.
**Conclusion:** Among persons with Type 2 diabetes without overt cardiovascular disease, a high risk for developing CHD (20–40 %) occurred in 35.8 % of them. The high risk for fatal CHD was still significant (17.4 %). A high risk for stroke occurred at 20 % of the patients. As a practical consequence, a complex clinical management, targeting concomitantly all the cardiovascular risk factors should be applied in order to reduce the cardiovascular risk.

## 1205

**Patient characteristics and glycemic control among patients with Type 2 diabetes mellitus with and without dyslipidemia**

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**Background and aims:** Type 2 diabetes mellitus (T2DM) is a well-known risk factor for development of dyslipidemia, most commonly manifested among T2DM patients as decreased HDL and elevated triglycerides. However, the prevalence of diabetic dyslipidemia and the specific patient characteristics associated with this condition have not been well described.
**Materials and methods:** Pharmacy and medical claims and laboratory data from a managed care organization with members in California, Arizona, and Colorado were used. Patients with T2DM (ICD-9-CM: 250.x0, 250.x2) were identified between July 1, 2000 and June 30, 2003. Dyslipidemia was

then identified based upon the following criteria: a lipid value indicative of dyslipidemia, i.e., low HDL (males: < 40 mg/dL; females: < 50 mg/dL), high LDL (LDL > 100 mg/dL), or high triglycerides (triglycerides > 150 mg/dL); a diagnosis of dyslipidemia; or a prescription fill for lipid-lowering therapy (LLT). Patients' index date was the first date of dyslipidemia or T2DM following their initially identified T2DM diagnosis. Patients were excluded if they were < 18 years old or were not continuously enrolled in the health plan for 180 days prior to this date (i.e., pre-index period). Clinical characteristics and prescription utilization during the pre-index period were compared between T2DM patients with and without dyslipidemia.
**Results:** A total of 93,359 T2DM patients with dyslipidemia and 45,305 without dyslipidemia were identified. Mean age of these patients was 66 ± 14 years, and males and females were evenly distributed. T2DM patients with dyslipidemia were significantly more likely than those without dyslipidemia to have hypertension (HTN; 42% vs. 37%) and coronary artery disease (CAD; 21% vs. 15%). Patients with dyslipidemia were significantly more likely to have an A1C < 7% (47% vs. 40%) and less likely to have neuropathy (9% vs. 10%) but were more likely to have retinopathy (7% vs. 5%). Patients with dyslipidemia were significantly more likely to have received sulfonylureas (43% vs. 40%), metformin (37% vs. 28%), and thiazolidinediones (10% vs. 5%). Patients with dyslipidemia were also significantly more likely than those without dyslipidemia to have received ACE inhibitors (43% vs. 32%) and angiotensin II receptor antagonists (AIIRAs; 6% vs. 3%). All comparisons between cohorts were significant at p < 0.0001. Among patients with identified lipid abnormalities (n = 37,289), the most common combinations of abnormalities were low HDL, high LDL, and high triglycerides (27%) and high LDL only (20%). HMG-CoA reductase inhibitors were the LLT used most often, although 61% of these patients received no LLT.
**Conclusion:** A substantial proportion of T2DM patients within this managed care population had evidence of dyslipidemia. Although diabetic dyslipidemia is typically associated with more progressed T2DM, patients in this population with dyslipidemia appeared to have less progressed disease, as evidenced by a greater likelihood of being at A1C goal. In contrast to other reports in the literature, T2DM patients within this population often had increased LDL, as well as low HDL and high triglycerides. Not surprisingly, two conditions that often go hand in hand with diabetic dyslipidemia, HTN and CAD, were more common among patients with dyslipidemia. ACE inhibitors and AIIRAs appeared to be underused within this population.
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## 1206

**Serum glucose as an independent predictor for mortality in non diabetic patients following acute myocardial infarction**
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**Background and aims:** Cardiovascular disease is the major cause of death in both diabetic and non diabetic patients in the western world. The Framingham study has documented a 2 to 4 fold increase in the risk of coronary heart disease in diabetics compared to non diabetic patients. Following an acute myocardial infarction diabetic patients have a significantly greater mortality than non diabetic patients in the short and long term. Both the Helsinki policeman and Whitehall studies have demonstrated that impaired glucose tolerance in non diabetic patients increases the risk of coronary heart disease but its impact on post-MI mortality is unknown. We have retrospectively studied whether admission glucose in non diabetic patients influences short (28 days) and long (500 days) term mortality after acute myocardial infarction.
**Materials and methods:** Data were collected from 768 consecutive non diabetic patients presenting to a single centre, Hope Hospital, Salford, UK from June 2000 to August 2003 with an acute myocardial infarction (AMI). AMI was defined as per ACC/ESC guidelines and and a threshold troponin T > 0.2umol /l was used. Patients with admission creatinine >200umol/l were excluded from study.
**Results:** In 469 patients admission glucose was <8 mmol/l, in 152 patients was 8–10 mmol/l and in 128 patients was >10 mmol/l. Patients with admission glucose between 8 and 10 mmol/l (n=152) and >10 mmol/l (n=128) had higher 28-day mortality than patients with admission glucose <8 mmol/l (n=469) (25.9%, 28.9%, 13.2%; p<0.001) After adjusting for age, troponin level and secondary prevention medication (Aspirin, B-Blockers, ACE inhibitors and Statins) the relative risk of death within 28 days was 1.64 (1.09–2.48) and 1.79 (1.16, 2.77) for 8 to 10 mmol/l and >10 mmol/l, respectively. Mortality at 500 days was also higher among patients with glucose between 8 and 10 mmol/l and >10 mmol/l than patients with admission glucose <8 mmol/l (37.5%, 42.2%, 23.7%; p<0.001). After adjusting for age, sex and troponin level and secondary prevention medication, the relative risks were 1.57 (1.13–2.18) and 1.52 (1.08, 2.15), respectively.
**Conclusion:** In conclusion this study suggests that following acute myocardial infarction, glucose is an independent predictor of short and longer term mortality in non diabetic patients. The effect appears to be independent of differences in age, sex, troponin level and secondary prevention medication. Intensive metabolic management with DIGAMI regime post AMI in patients with an admission glucose >11.1 mmol/l improves outcome. It should be investigated whether this approach should be extended to the higher risk group with admission glucose 8–11.1 mmol/l.

## 1207

**Hyperglycemia during acute myocardial infarction in diabetic and nondiabetic patients**
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**Background and aims:** Hyperglycemia is a risk factor for increased mortality after acute myocardial infarction (AMI) in diabetic patients. The aim of this study was to evaluate the frequency and the relationship between hyperglycemia and the risk of poor outcome and death in a cohort of patients with AMI.
**Materials and methods:** The data from 346 AMI patients medical records (213 men, 133 women) , mean (+/–SD) age 66 +/– 12.3 years, 70 with previously known diabetes (KD), successively admitted to coronary care unit during an entire year, were used. According to fasting (FPG) and random (RPG) plasma glucose levels during the first 24 hours, the patients without previously known diabetes (n=276) were classified as having diabetic hyperglycemia (DH: FPG >/=7.0 and/or RPG >/= 11.1 mmol/l; n=81), prediabetic hyperglycemia (PH: FPG >/=6.1 but < 7.0 and/or RPG >/= 7.8 but < 11.1 mmol/l; n=84) or normoglycemia (NG: FPG < 6.1 and RPG < 7.8 mmol/l; n=78). Thirty three patients with normal (<7.8 mmol/l) RPG levels but without FPG determination were not included in this classification.
**Results:** Together, KD, DH and PH groups accounted for 68% from total AMI cases. During hospitalization, 64 patients (18.5%) had died. As a group, compared to survivors, they had greater age (71.7 +/– 10.3 vs 65.0 +/– 12.5 years; p < 0.001), FPG (8.7+/–4.3 vs 7.0 +/–3.0 mmol/l; p < 0.002) levels and lower left ventricular ejection fraction (LVEF: 34.5%+/–12.3 vs 46.3%+/–10.4; p < 0.001); there were no significant differences in serum lipid levels.The greatest in-hospital mortality rate, as compared to NG (6.4%), was observed in DH group (29.6%; RR = 4.6; p < 0.001) followed by KD (18.6%; RR = 2.9; p < 0.03) and PH (13.1%; RR = 2.04; p = 0.15) group. Between men and women in KD (19.1% vs 17.4%) and DH ( 29.2% vs 30.3%) groups it was no significant difference in death rate. Between KD, DH, PH and NG groups there were no significant differences in age, serum HDL-cholesterol and total cholesterol mean levels. KD group had greater frequency (80.3% vs 62.8%; p < 0.03) of arterial hypertension, increased levels of serum triglycerides (2.05 +/– 1.6 vs 1.48 +/– 0.96 mmol/l; p < 0.02) and DH group had greater serum AST levels (270.5 +/– 383 vs 178.9 +/– 124.5 u/l; p < 0.04) than NG group. PH (46.2%+/–9.9 ; p < 0.03), DH (43.3%+/–12.7; p < 0.003) and KD (41.3%+/–11.8; p < 0.001) groups had lower LVEF as compared to NG (49.8%+/–8.5) group. A significant, independent, negative correlation between LVEF, FPG (r = –0.24; n = 256; p < 0.001), RPG (r = –0.27; n= 256; p < 0.001) and age (r = –0.22; n= 256; p < 0.001) was observed.The risk of death (RR = 2.01; n = 108; p < 0.003) was increased in patients with (compared to 238 patients without) an antecedent stroke (AS) and/or an antecedent myocardial infarction (MI). The patients with (compared to patients without) AS and/or antecedent MI had greater age (69.7+/–10.7 vs 64.9+/–12.8 years; p=0.001) and lower LVEF (41.0%+/–10.5 vs 47.7%+/–10.3; p<0.001) but no differences regarding serum lipids and blood glucose mean values.
**Conclusion:** Hyperglycemia, a frequent encounter during AMI, was significantly associated to risk of poor outcome and death. Hyperglycemia was inversely, and independent of age, correlated to LVEF. After AMI, the patients without previously known diabetes with DH and the patients with an antecedent stroke and/or an antecedent myocardial infarction had the greatest inhospital mortality.

## 1208

**The Munich Myocardial Infarction Registry: glucose-insulin-infusion in a clinical setting**

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**Background and aims:** The Myocardial Infarction Registry of the Academic Schwabing City Hospital analyses morbidity and mortality in diabetic (D) and non-diabetic (ND) patients. In 2001, administration of glucose-insulin-(GI)-infusion in D has been introduced for early metabolic intervention. The aim of the present study was to assess D with and without glucose-insulin infusion and to compare them with regard to hospital course and other therapeutic interventions.
**Material and methods:** All diabetic patients (known diabetes or blood glucose > 180 mg/dl on admission) with acute myocardial infarction (AMI), who were admitted to intensive care unit between 2001 and 2003, were assessed. D, who received infusion with glucose and regular insulin were compared to those, who were treated conventionally.
**Results:** 380 (39%) of 988 patients with AMI were classified as D. GI-infusion was applied in 189 (50%) D at a dose of 2.0 ± 3.1 IE/h for 32 ± 36 hours (mean + SD). HbA1 c was 7.6 ± 1.6% in the infusion group compared to 7.1 ± 1.6% in the non-infusion group ($p<0.01$). Diabetes duration was 6.1 ± 7.8 yr. in the infusion group and 4.0 ± 8.4 yr. in the non-infusion group ($p<0.001$). In the infusion group, blood glucose levels decreased from 209 ± 79 mg/dl to 161 ± 59 mg/dl ($p<0.001$) after 24 hours and to 145 ± 49 mg/dl ($p<0.001$) at the end of GI-infusion. In patients with GI-infusion, severe hypoglycaemias did not occur. Mortality within 24 hours after admission was 2% in the infusion-group compared to 12% in the non-infusion group ($p<0.001$). Total hospital mortality was 14% compared to 28% ($p=0.001$).
Therapeutic interventions were performed more frequently in the infusion-group compared to the non-infusion group: coronary angiography (80% vs. 59%; $p<0.001$), GPIIb/IIIa-RA (65% vs. 37%; $p<0.001$), PTCA (55% vs. 35%; $p<0.001$), coronary stenting (59% vs. 40%, $p<0.001$). In the infusion group, catecholamines and resuscitation were applied less frequently (17% vs. 27%, $p<0.05$; 11% vs. 18%; $p<0.05$).
**Conclusion:** The study demonstrates that glucose-insulin-infusion can be successfully introduced in a clinical setting. It emphasizes that in a subset of diabetic patients without insulin infusion, an intensification of interventions targeting both metabolic control and arterial occlusion is required.

# PS 124

# Cardiac complications: epidemiology and prognosis

## 1209

**Diabetes and acute myocardial infarction: is the prognosis changing? Surprising data from the Reggio Emilia province (Italy)**

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**Background and aims:** The province of Reggio Emilia, in northern Italy, with a resident population of 462.858, has a cardiologic network based on a hub in the hospital of Reggio Emilia – where coronaroangiography and Percutaneous Coronary Intervention (PCI) are performed (primary, facilitated or rescue) – and spoke Intensive Coronaric Units (ICU) in five peripheral hospitals, where first aid and thrombolytic therapy are performed. Aim of the study was to verify the prognosis of Diabetics (D) vs non-Diabetics (C) with Acute Myocardial Infarction (AMI).
**Materials and methods:** All clinical data of AMI are reviewed in a clinical audit held at the end of each year. Data from 2000 and 2001 files are reported.
**Results:** A cohort of 1075 AMI were admitted in the period: 318 were females and 757 males, and 202 out of 1075 (18,7%) were known (and verified) as diabetics. The F/M ratio in diabetics (79/123) was significantly higher (0.02). Females (74 +/–13yrs) were significantly older than males (65+/–13yrs), independently of the presence of diabetes (0.0001). The history of previous AMI of angina or revascularization was not different between controls and diabetics . The time interval from onset of symptoms to arrival in the ICU was higher in D (only 13.9 % were admitted in the first hour vs. 18.5% in C). D had lower levels of cardiac enzymes (troponin, CK and CK activity), while the site of AMI at the electrocardiogram was similar, with the exception of a higher prevalence of undefinable site of the lesion in D. Thrombolysis with rt-PA was performed in an equivalent number of subjects (28,0 in C and 23,7% in D) with ST elevation (STEMI), was successful in > 80% of them, and mortality was very low (2,3%). In the acute phase of AMI C and D experienced a similar number of clinical complications (angina, re-infarction, acute heart failure, arrhythmias), but only ventricular fibrillation was observed significantly more frequently in C than in D. Totally 446 pts (41.4%) – with ST elevation – were transferred to the central hospital and underwent coronary angiography. The number of vessels with a stenosis >75 % was significantly higher in D than in C (0.05). Primary or facilitated PCI was performed in 18,5% of pts and was increasing from 2000 (14,1%) to 2001 (24.7%); PCI was performed slightly more frequently in C (19,3) vs in D (14,8 %). In 62.2% of C and 54.0% of D the ECG pattern on discharge from hospital showed a Q-wave AMI (STEMI) while in 37.8% of C and 46% of D (0.03),there was a non-Q wave AMI (NSTEMI). In the 87,5% of pts, a 2D echocardiogram was performed before discharge: an EF > 35% was observed in 81,8% of them, independently of the presence of diabetes. The in-hospital mortality was similar in D than in C (3,9% vs 7,6%, ns); also at 30 days mortality was similar both in D and C (6,9 vs 9.5%, ns).
**Conclusion**: The setting of a cardiological network covering the Reggio Emilia area is improving the clinical outcome of AMI with a decrease in mortality from 9.1 in 2000 to 6.4% in 2001, with a further decrease from 1999 (12%), when there is'nt network.We observed a lower mortality from AMI in diabetic pts: the extensive use of intensive insulin treatment and of glycoprotein IIb-IIIa inhibitors in D along with the increased invasive treatment with PCI, could probably account for that surprising result.

## 1210

**Silent myocardial ischemia prognosis: a six year follow-up of 203 diabetic patients**

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**Background and aims**: The aim of this study is to evaluate the prognosis of a cohort of type 1 and type 2 diabetic patients, 6 years after a silent myocardial ischemia (SMI) screening, and to define predictive factors of this prognosis.

**Materials and methods:** Initially, two-hundred and three asymptomatic diabetic patients underwent a systematic SMI screening : electrocardiogram exercise test and/or a thallium-201 myocardial scintigraphy and coronary angiography if, at least, one of the aforementioned was positive. This screening allowed to define three groups; group 1: patients (n = 170) with functional negative screening; group 2: patients (n = 33) with positive screening according to at least one positive functional test; and group 3: patients (n = 21) with positive screening and coronary stenoses. Stenosis was scored in term of percentage of cross-sectional narrowing; coronary stenosis of ≥ 50 % was considered significant. Six years after the initial screening, all patients were asked to attend a reffered clinical examination, a rest ECG and cardiologic functional tests when necessary. All events (death, cardiac death, non fatal major cardiac events) were recorded. Statistical analyse tests were the $\chi^2$ test and stepwise logistic regression models.
**Results:** Fifteen patients were lost for follow-up, 14 in group 1 and one in group 2. During 6 years, patients of group 3 (n = 20) with a positive initial SMI screening and significant coronary stenosis have a very high risk of non fatal major cardiac events when compared to patients of group 1 (n = 156) with a negative initial screening : 35 % vs 7 %, $p < 0.001$. The whole mortality of patients of group 3 is higher when compared to patients with a negative screening (group 1; 35 % vs 15 %, $p < 0.05$ ). Patients of group 2 (n = 32) have a significantly higher non fatal major cardiac events rate compared to negative SMI screening patients (group 1); mortality rate does not differ significantly between groups 1 and 2. Cardiac death and death from all causes are not significantly different among the three groups; cancer is the first cause of death (36.4 %). In our whole population, univariate analysis show that major cardiac events are significantly related with age ($p < 0.05$), body mass index ($p < 0.05$), hypertension ($p < 0.05$), the existence of carotid atherosclerosis ($p < 0.05$), total cholesterol ($p < 0.05$) and triglycerids ($p < 0.05$) levels and with coronary stenoses ($p < 0.001$). In multivariate analysis, major cardiac events are only correlated with age, body mass index and coronary stenoses ($p < 0.01$).
**Conclusions:** This study confirms the very poor prognosis of SMI in diabetic patients (cardiac events or death), especially when they have coronary stenoses. Taking all cardio-vascular risk factors into account as intensively and as soon as possible in the treatment is mandatory for these high risk patients. Neverless, in our diabetic population, cancers and not heart diseases represent the first cause of death.

## 1211

### Dynamics of epidemiological indices of ischemic heart disease in the cohort of Type 2 diabetic persons from defined population – 10-year follow-up

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**Background and aims:** Ischemic heart disease (IHD) is the most frequent macroangiopathic complication and the main cause of death in persons with diabetes mellitus type 2. The epidemiological dynamics of this fact is shaped by the interaction of time and of specific complex of risk factors acting in defined population. For planning the practical, preventive programmes it is necessary to delineate its local character. Therefore the study of the significance of this assumption by analysing the changes in morbidity and incidence of IHD and myocardial infarction (MI) in a representative cohort of diabetic persons type 2, remaining during 10-year period in the same life and therapeutic defined environment, was undertaken.
**Materials and methods:** A representative cohort of 1334 type 2 diabetic persons recruited from the out-patient clinic operating only in the defined environment had been primarily examined in January 1992. The diagnosis of IHD and MI was established on the base of: 1) changes in ECG according to Minnesota code (1.1–1.3, 3.1–3.3, 4.1–4.3, 7.1) or/and 2) clinical symptoms and 3) discharge information sheets. During the 10-year follow-up the transformations in the IHD epidemiology and the concomitant risk factors – age, duration of diabetes, BMI, fasting and postprandial glycemia, plasma cholesterol and triglycerides, serum creatinine, 24-hour proteinuria, smoking and arterial blood pressure have been continuously studied and their impact on epidemiological indices assessed.
**Results:** At baseline (1992) in the total cohort among type 2 diabetic persons 475 subjects (35.6%) had suffered from IHD. The subgroup without IHD at baseline was formed by 859 of 1334 persons (64.4%). During the analyzed 10-year period in this fraction of the cohort 405 new cases of IHD have been diagnosed (47.1%). The incidence of IHD was 80.6 per 1000 person-years. Mean age at the moment of diagnosis of new IHD was 66.6 ± 9.4 years and mean duration of diabetes mellitus was 12.1 ± 6.0 years. In the whole cohort under study 240 events of MI have been found in the monitored 10 years. Among 1108 from 1334 persons of the entire cohort without MI before 1992, 157 experienced one new MI, 18 – two new MI and 1 – three new MI during 10-year follow-up. The morbidity and incidence of new case of MI was respectively 16.9% and 22.5 per 1000 person-years. In 55 cases the first MI was the cause of death. In a group of 226 subjects after MI before 1992 – 39 persons experienced next MI. In 24 persons these infarctions were the cause of death. The logistic analysis showed, that incidence and morbidity due to IHD and MI was associated mainly with hyperglycemia, hypertension and the level of therapeutic intensity.
**Conclusion:** The continuous monitoring of the epidemiological indices of IHD and MI in defined type 2 diabetic population by delineating their dynamics permits to assess the intensity and the composition of atherogenic risk acting in the specific environmental and therapeutic conditions. The regular epidemiological monitoring of IHD and MI in defined population is indispensable for better planning and implementation of practical prevention which is always addressed to the defined populations.

## 1212

### The effect of moderate alcohol consumption on long-term prognosis in Type 2 diabetics following acute coronary syndromes

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**Background and aims:** It is well established that moderate alcohol consumption (1–2 alcoholic beverages per day) (MAC) is associated with better long-term prognosis in patients with coronary artery disease. However, the effect of MAC on the long-term prognosis following acute coronary syndromes in type 2 diabetic pts has not thoroughly evaluated.
**Materials and methods:** A total of 377 consecutive type 2 diabetic pts, who admitted in our institute in the first 24 hours of the onset of an acute coronary syndrome, were studied. Pts were followed-up for up to five years and cardiovascular mortality was the primary endpoint. Patients were divided into 3 groups according to the amount of alcohol consumption during the follow-up. Group I: no or rarely (138 pts); Group II: 1–6 alcoholic beverages per week (126 pts); and Group III: 1–2 alcoholic beverages per day (MAC) (113 pts).
**Results:** There were no significant differences in baseline characteristics among the study groups. During the follow-up period the incidence of cardiovascular mortality was significantly lower in type 2 diabetic pts who drank moderately (40.3%; 39.6%; and 22.4 for groups I; II; and III, respectively; p=0.001).
**Conclusion:** The results of the present study suggest a beneficial effect of MAC on long-term cardiovascular mortality in type 2 diabetic pts following an acute coronary syndrome.

## 1213

### Prognostic significance of diabetes mellitus in an outpatient population assisted in a Heart Failure Unit: one year mortality and hospital admissions due to heart failure

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**Background and aims:** Although diabetes mellitus (DM) is well recognised as a major risk factor for cardiovascular disease, its relationship with heart failure (HF) is not fully established. The aim of the study was to asses the prevalence of DM in an outpatient population with HF assisted in a HF Unit and to evaluate the prognostic significance of DM considering one year mortality and hospital admissions due to HF during the first year of follow-up.
**Materials and methods:** Between August-2001 and March-2003, 341 patients have been admitted to the Unit. Survival status and HF related hospital admission rate at 1 year have been available in 337 patients (72% men, mean age ± SD, 68.4 ± 11 years). Demographic, clinical and laboratory data were recorded at the first visit to the Unit. Aetiology of HF was: ischemic heart disease 59%, dilated cardiomyopathy 11%, hypertensive cardiomyopathy 8%, alcoholic cardiomyopathy 6%, valvular disease 7%, and other 9%. Fifty-five percent of patients have history of previous myocardial infarction. Mean ejection fraction was 31.6 ± 12% and median time since HF symptoms onset was 26 months (range 0–288). Mean hospitalisations due to HF during the preceding year were 0.85 ± 1.8 (range 0–15). Patients were in NYHA functional class I (5%), II (46%), III (44%) and IV (5%). Chi-square test and multiple lineal regression were used for statistical analysis.

**Results:** One-hundred thirty-seven out of 337 patients were diabetic (41%). Twenty-eight patients (8%) died and there were 158 hospital admissions due to HF in 66 patients during the first year of follow-up. One year mortality was 5% in non-diabetic patients and 13% in diabetic patients (p=0.008). A 13.5% of non-diabetic patients suffered at least one HF related hospital admission, whereas 28.5% of diabetic patients needed to be hospitalised at least once due to HF (p=0.001). In the multivariate regression analysis apart from age, sex, NYHA functional class, LVEF, and number of hospitalisations during the preceding year, DM remained statistically associated with the need of HF related hospital admission. One year mortality, however, correlated more strongly with other parameters such NYHA functional class, number of HF hospitalisations during the preceding year and plasmatic haemoglobin levels.
**Conclusion:** Prevalence of DM in a general population with HF was high and significantly correlated with one year mortality as well as with the need of hospital admission due to HF during the first year of follow-up. However, other parameters, such NYHA functional class, number of HF hospitalisations during the preceding year and haemoglobin plasmatic levels had a higher influence on mortality.

## 1214

**Prevalence of *unknown* diabetes and other cardiovascular risk factors in patients before cardiac transplantation**

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**Background and aims:** Diabetes mellitus (DM), high blood pressure (HBP) and dyslipidaemia (DL) are *major* independent cardiovascular risk (CVR) factors and well-known causes of morbidity and mortality for cardiac transplantation (CT). In many cases, CT is indicated in patients with advanced coronary heart disease. These CVR factors are frequently detected at the same moment that the patient is included in the CT program. The aim of this study is measure the actual and the *unknown* prevalence (unknown = actual - *known*) of DM and others CVR factors in patients before a CT.
**Materials and methods:** We collected the data of the study protocol pre- CT from 1996 until 2003: n = 65 (5 women, 60 men). It includes: Oral glucose tolerance test (OGTT), plasma lipids and uric acid determinations, body weight and height measures and a questionnaire about previous diagnosis of DM, HBP, DL, hyperuricaemia (HU), smoking and pharmacological treatment.
**Results:** Data expressed as *known vs* actual prevalence: DM (24.6 *vs* 56.9 %), DL (38.2 *vs* 85.7 %), HBP (21.5 *vs* 46.8 %), HU (28.1 *vs* 63.1 %). Impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG) prevalence: 21.5 %. Only 46 % of patients with known DL were under pharmacological treatment.
**Conclusion:** The prevalence of *unknown* DM, DL, HBP and HU in patients before CT is too high: 32.3, 47.5, 25.3, and 35 %, respectively. The prevalence of IGT+IFG was 21.5 %, resulting in a total prevalence of abnormal glucose tolerance (IFG+IGT+DM) of 78.4 %. In this group of patients, with a high CVR score, before submission to a risky surgical procedure and under specialised medical supervision, the diagnostic and therapeutic effort in DM and other CVR factors should be improved.

## 1215

**Effect of influence of treatment by various antidiabetic agents on lethality and mortality in patients with acute coronary syndrome and Type 2 diabetes mellitus**

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**Background and aims:** to estimate effect of treatment by insulin, gliclazide, glibenclamid with glycemia maintenance within 24 hours in the range of 4,0 - 7,7 mmol/l in patients with acute coronary syndrome (acute myocardial infarction - AMI) and instable angina - IA), suffering from Type 2 diabetes mellitus (Type 2 DM) on lethality and mortality within 1 year after discharge from hospital.
**Materials and methods:** 152 patients with TYPE 2 DM and AMI with wave Q (62 men and 90 women) and 299 patients with TYPE 2 DM and IA (127 men and 172 women) aged 42–75 (mean age 58 ± 1,7) took part in the open research. The patients received treatment at cardiological department of the Krasnodar city center of emergency hospital in 2001–2002 y. TYPE 2 DM duration was from 5 months to 27 years (mean 9,7 ± 0,2 years). All groups were comparable in sex, age, duration of TYPE 2 DM, glycemia 4,0 - 7,7 during 24 hours. Patients having been hospitalized, metformin was cancelled. Patients continued treatment with gliklazide-80 or glibenclamid if glycemia stably kept in range 4,0,-,7,7 mm/L. Patients with blood glucose 7,8 mm/L and higher were transferred to insulin during first three days after their admission to hospital. Patients were divided into 6 groups: the patients taking simple insulin intravenously dropwise within first three days after in admission to hospital with the subsequent transition to 2–3 injections of simple insulin and if necessary an injection of prolonged insulin before dream sleep 1 year after a discharge from the hospital (I group - 32 people with AMI, IV group - 47 people with IA); the patients receiving gliclazide-80 mg from 1 up to 3 tablets per day in the hospital with transition to gliclazide - SR after the discharge (II group - 58 people with AMI, V group - 146 person with IA); the patients receiving glibenclamid (III group - 62 persons with AMI, V group - 106 people with IA). Lethality, mortality were estimated within 1 year after the discharge. Correlation and criterion Student are calculated with the help of statistical program 6,0. Distinctions were recognized authentic at $p < 0,05$. Examination, glycemia and lipid control, blood pressure, electrocardiogram were made in the hospital in 1, 3, 12 months after discharge. Talks on carefulness fulfillment of all medical recommendations were carried out with each patient.
**Results:** in groups I, II, and III lethality and mortality were authentically higher, than in groups IV, V, VI ($p < 0,001$). Among patients in I, II, III groups lethality was lower in I group to group in comparison with groups II and III without an authentic difference ($p = 0,05$). At comparison of mortality in groups with AMI the given parameter correlated with duration of diabetes with the degree of expressiveness of chronic heart failure, presence of arrhythmia, but not with a kind of antidiabetic agents.
**Conclusion:** The kind of antidiabetic agents does not influence lethality and mortality in 1st year after discharge from the hospital if glycemia is maintained in a range 4,0 - 7,7 mm/l and a patient accurately follows doctor's prescriptions on preventive maintenance and treatment from hyperlipidemia, arterial hypertension, chronic heart failure.

# PS 125

# Macrovascular disease: cellular mechanisms in animals

## 1216

**Late intervention with pyridoxamine retards progression of atherosclerosis in diabetic apoE knockout mouse**

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**Background and aims:** Advanced glycation end products (AGEs) have been suggested to play a central role in the development and progression of diabetic micro-and macrovascular complications. Pyridoxamine (PYRI) is an inhibitor of the formation of AGEs and advanced lipoxidation end products (ALEs). We aimed to investigate the effects of PYRI in the diabetic apoE KO mice, a model of accelerated atherosclerosis.
**Materials and methods:** ApoE KO mice were rendered diabetic by 5 daily streptozotocin injections (55 mg/kg daily, ip) at 6 weeks of age. At week 10 of diabetes animals were randomised to receive no treatment or PYRI (1g/L water). At week 20 of diabetes animals were sacrificed and blood and aorta collected for further analysis.
**Results:** Pyridoxamine prevented the progression of established atherosclerosis in diabetic mice with no effect on lipids or on glycaemic control. The reduction in plaque area was associated with reduced expression of the AGE receptor RAGE, the pro-fibrotic growth factor TGF-beta, and alpha-SMA positive cells, as well as a reduction in the pro-angiogenic growth factor VEGF, at the gene and protein level.
**Conclusion:** These results support the pivotal role for AGEs in diabetes related atherosclerosis and demonstrate retardation with pyridoxamine treatment.

| Results | Control | Control+ PYRI | Diabetic | Diabetic+ PYRI |
|---|---|---|---|---|
| GHb (%) | 3.1 ± 0.2 | 4.4 ± 0.1 | 19.3 ± 0.5** | 16 ± 0.7*# |
| Body Weight (g) | 30.9 ± 0.8 | 30.6 ± 0.4 | 21.8 ± 0.5* | 22 ± 0.5* |
| S. Cholesterol (mM) | 16.6 ± 1.3 | 15.5 ± 1.5 | 28.8 ± 1.7** | 28.3 ± 1.6** |
| Plaque Area (%) | 3.7 ± 0.9 | 1.5 ± 0.3* | 11.8 ± 1.1* | 5.7 ± 1.0# |
| RAGE protein (5) | 2.5 ± 0.6 | 1.9 ± 0.6 | 7.0 ± 0.6** | 3.3 ± 0.7# |

** $p < 0.01$, * $p < 0.05$ vs control, ## $p < 0.01$, # $p < 0.05$ pyridoxamine vs diabetic

*Supported by: Juvenile Diabetes Research Foundation*

## 1217

**Correlation of increased superoxide production with expression of eNOS and COX II in the vasculature of diabetic Goto-Kakizaki-Rats**

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**Background and aims:** Diabetic vascular complications involve oxidative stress. This oxidative stress can be mediated by reactive oxygen species such as peroxynitrite and superoxide anions ($O_2^{-}$). The aims of this study were to show oxidative stress in vascular tissues and identify sources of vascular $O_2^{-}$ production in a non-obese non-hypertensive animal model of type II diabetes, the Goto-Kakizaki (GK) rat.
**Materials and methods:** Hyperglycaemia was confirmed in 16-week old male diabetic rats by blood glucose measurements. Mesenteric artery rings, 2 mm in length, were isolated from 12-week old normal Wistar (control) or diabetic animals for measurement of endothelium dependant (acetylcholine, ACh, 0.001–30μM) relaxation (EDR) by isometric force displacement, in tissues pre-contracted with 0.1μM phenylepherine. $O_2^{-}$ levels in mesenteric artery branches were measured by lucigenin (5μM) chemiluminescence and expressed as arbitrary units (au)/mg wet wt tissue. Western Blot analysis from aortic tissue was carried out on 7–15% resolving gels as appropriate and blots were probed for eNOS, Cu/Zn-superoxide dismutase (SOD), cyclooxygenase II (COX II) and nitrotyrosine (NT) (all blots normalised to β-actin). Data were analysed using ANOVA or unpaired t-tests as appropriate and a p-value <0.05 was taken to indicate significance.
**Results:** At 16 weeks diabetic blood glucose levels (mM) were significantly higher than their age-matched Wistar controls (13.8 ± 0.9 vs. 6.7 ± 0.2, $p<0.001$). Diabetic rats had impaired EDR compared with age-matched controls ($R_{max}$, maximal relaxation 41.5 ± 2.8%, vs. 83.8 ± 5.7%, respectively $p<0.05$, n=5–7). There was an increase in basal $O_2^{-}$ production in diabetic vessels (31.3 ± 9.5 vs. 92.9 ± 24.9 au, $p<0.05$), but neither NADPH (100 μM) nor NADH (100μM) increased $O_2^{-}$ production in either vessel type (control: basal vs. NADPH: 60.9 ± 38.9 vs. 195.3 ± 25.98 au; diabetic: basal vs. NADPH: 25.8 ± 6.9 vs. 286 ± 99; control: basal vs. NADH: 51.9 ± 48 vs. 410 ± 142; diabetic: basal vs. NADH: 0.079 ± 0.01 vs. 423 ± 193, n=5, p=ns). In contrast, the flavoenzyme inhibitor diphenyleneiodonium chloride (DPI, 100 μM) alone significantly raised $O_2^{-}$ in all cases (control: basal vs. DPI 51.9 ± 48 vs. 969 ± 306; diabetic: basal vs. DPI: 25.8 ± 6.9 vs. 712 ± 205, n=5, $p<0.05$). Protein expression levels of COX II, eNOS and NT were all increased in diabetic tissues (% control COX II: 142.4 ± 13.6, eNOS: 234.8 ± 45.1, NT: 156.3 ± 24.9, all $p<0.05$, n=4–6). Levels of SOD were not significantly different in control and diabetic tissues.
**Conclusion:** Evidence for endothelial dysfunction in the vasculature of GK rats is supported by increased basal $O_2^{-}$ production and NT expression in diabetic tissues. The increases in COX II and eNOS expression is suggestive of their contributing to oxidant stress, since uncoupling of both enzyme systems can lead to excessive $O_2^{-}$ generation at the expense of physiological mediators such as prostacyclin and nitric oxide respectively.
*Supported by the Irish Health Research Board*

## 1218

**The free fatty acids (FFAs) selectively inhibit Akt-mediated eNOS phosphorylation at Ser1177 in rat aortic endothelial cells in vivo**

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**Aims:** Elevated FFAs levels have been shown to impair endothelium dependent vasodilation (EDV) which may related with the increased risk of macrovascular disease in insulin resistant patients. Here we test the hypothesis that acute elevation of free fatty acids induced endothelial dysfunction may caused by inhibiting Akt-eNOS-NO signaling pathway in rat aortic endothelial cells.
**Materials and methods:** Male S-D rats underwent 6 hr infusion in two groups: Control (C, n=30): infused with normal sailine; FFA (n=30): infused with FFAs (20%intralipid + heparin). At the end of infusion aortas were harvested and cleaned of adhering tissue and blood inside the lumen. The endothelial cells were gleaned by scraper and solubilized by sonication in homogenizing buffer. The serine phosphorylation and protein levels of Akt and eNOS in the cell lysate were determined by western blotting. The tyrosine phosphorylation and protein levels of p44/42 MAP kinase (ERK-1/2) were detected by same method. The plasma FFA levels were determined by an enzymic method.The analysis of serum nitrite/nitrate (NOx) was performed by HPLC.
**Results:** The results showed that in FFA group the plasma FFAs concentration were higher (FFA: 1146.9 ± 223.1 vs. C: 327.3 ± 106.5 micro-mol/L, $p < 0.01$) and the serum NOx concentration were lower (FFA : 12.6 ± 1.9 vs. C : 17.2 ± 3.9 micro-mol/L, $p < 0.01$). The level of Akt phosphorylation at Ser 473 in FFA group was decreased by approximately 21% compared with C group ($p<0.05$). The level of Akt-mediated eNOS phosphorylation at Ser1177 in FFA group was decreased by approximately 28%($p< 0.05$), there were not significantly different in the protein levels of Akt and eNOS in both groups ($p>0.05$).The tyrosine phosphorylation and protein levels of p44/42 MAP kinase were not significantly different in both groups ($p>0.05$).
**Conclusions:** The present study indicated acute elevation of free fatty acids directly and independently reduces endothelial NO production.The mechanism underline may related, at least partly, down-regulates some aspects of insulin signaling (Akt-mediated eNOS phosphorylation at Ser1177), whereas p44/42 MAP kinase signaling pathway was not altered in rat aortic endothelial cells. It is suggested that elevated FFAs level might play a crucial role in the endothelial dysfunction accompanied metabolic syndrome through selective inhibition of Akt-mediated eNOS phosphorylation at Ser1177.

## 1219

**Inhibition of NF-kB activity protects proliferation and PAI-1 expression induced by high glucose in vascular smooth muscle cells**

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**Background and aims:** Vascular smooth muscle cell (VSMC) dysfunction by high glucose is a characteristic of diabetic vascular complications.

Activation of Nuclear factor KB (NF-kB) may be a key player in the regulation of inflammation and proliferation of VSMC. We have examined whether VSMC proliferation and plasminogen activator inhibitor-1(PAI-1) expression induced by high glucose were mediated by NF-kB activation, and selective inhibition of NF-kB by expression of an IkB-alpha mutant (IkB-aM) would inhibit proliferation and PAI-1 expression in vascular smooth muscle cell.
**Material and methods**: VSMCs were cultured from thoracic aorta of male SD rats. VSMC proliferation was examined by MTT assay. PAI-1 expression was assayed by RT-PCR. NFkB acitivation was studied by immunohistochemical staining, immunoblotting by IkB antibody. VSMC infected with recombinant adenovirus vectors (Ad-IkBaM). After transfection, MTT, PAI-1 expression was retested.
**Results:** Glucose stimulated VSMC proliferation in dose dependent manner up to 400 mg/dL. But VSMC proliferation was not observed by mannitol given to deliver the same osmolar stress to the cells. PAI-1 expression was stimulated by glucose in dose dependent manner. High glucose (400 mg/dl) alone induced an increase of NF-kB activity in VSMC. Inhibitor of NFkB, MG132 inhibited the VSMC proliferation induced by high glucose. Expression of IkB-aM in VSMC led to reduction in glucose-stimulated cell growth and PAI-1 expression.
**Conclusion**: Inhibition of NF-kB activity protects proliferation and PAI-1 expression induced by high glucose in vascular smooth muscle cells.

## 1220

**NAD(P)H oxidase-derived superoxide mediates the enhanced expression of cell adhesion molecules in the aorta of diabetic mice**

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**Background and aims:** We performed this study to determine the relationship among vascular production of superoxide, expression of CAM and diabetes.
**Materials and methods:** We measured superoxide generation and expression of intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin in the aorta from control (C57BL/6J) and diabetic mice (ob/ob).
**Results:** In situ staining for superoxide using dihydroethidium showed a marked increase of superoxide production in diabetic aorta in association with an enhanced NAD(P)H oxidase activity. Immunohistochemical analysis revealed that the endothelial expression of ICAM-1 ($3.5 \pm 0.4$) and VCAM-1 ($3.8 \pm 0.3$) in diabetic aorta was significantly higher than that in control aorta ($0.9 \pm 0.5$ and $1.6 \pm 0.3$, respectively). Furthermore, there was a strong positive correlation ($r = 0.89$, $p<0.01$ in ICAM-1 and $r = 0.88$, $p<0.01$ in VCAM-1) between ICAM-1/VCAM-1 expression and vascular production of superoxide.
**Conclusion:** The present data indicate that the increased production of superoxide via NAD(P)H oxidase may explain the enhanced expression of CAM in diabetic vasculatures.

# PS 126

# Peripheral macrovascular disease

## 1221

**Brachial-ankle pulse wave velocity is correlated with coronary artery calcification in diabetic patients**

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**Background and aims:** Diabetic patients are at high risk of cardiovascular disease. Aortic stiffness is regarded as a cardiovascular risk factor, though, it is not ascertained whether aortic stiffness reflects coronary arteriosclerosis in diabetic patients. To evaluate the relationship between aortic stiffness and coronary calcification, we examined pulse wave velocity (PWV) and coronary artery calcification in diabetic and non-diabetic patients.
**Materials and methods:** We measured brachial-ankle PWV (baPWV) and ankle-brachial index (ABI) by form PWV/ABI (Colin Co. Ltd.), and coronary calcification score (CCS) by electron-beam computed tomography (EBCT) as an estimation of coronary arteriosclerosis in 423 of diabetic and 109 of non-diabetic patients (whose age ranged 45–75 years). Those who had low value of ABI (< 0.9) and high value of serum creatinine (> 1.1 mg/dl) were excluded from this study. Predicted PWV was obtained from the nomogram adjusted for age and systolic blood pressure. The difference between measured baPWV and predicted PWV was refered to ΔPWV.
**Results:** Measured PWV (cm/s) and ΔPWV (cm/s) were $1725 \pm 392$ and $244 \pm 294$ in diabetic patients and $1716 \pm 502$ and $198 \pm 438$ in non diabetic patients, respectively. Profiles of diabetic vs non-diabetic subjects were as follows; age (years): $61.6 \pm 7.2$ vs $63.2 \pm 7.7$, HbA1 c (%): $8.9 \pm 1.9$ vs $5.7 \pm 0.4$, CCS: $221 \pm 545$ vs $192 \pm 383$, HDL-choleterol (mg/dl): $56 \pm 17$ vs $59 \pm 18$, systolic blood pressure (mmHg): $131 \pm 20$ vs $133 \pm 20$ and diastolic blood pressure (mmHg): $77 \pm 11$ vs $78 \pm 12$. By multiple regression analysis, ΔPWV was significantly correlated with HbA1 c ($\beta=0.13$, $p<0.01$), age ($\beta=0.13$, $p<0.01$) and CCS ($\beta=0.13$, $p<0.01$) but not systolic blood pressure, HDL-cholesterol or sex in total subjects. In diabetic patients, ΔPWV was significantly correlated with age ($\beta=0.16$, $p<0.01$), CCS ($\beta=0.13$, $p<0.01$), and in non-diabetic patients, ΔPWV was correlated with neither age, sex, CCS, systolic blood pressure nor HDL- cholesterol.
**Conclusion:** baPWV was a useful indicator reflecting coronary artery calcification in diabetic patients, but not in non-diabetic patients.

## 1222

**The usefulness of toe brachial pressure index using a new developed oscillometric method in patients with diabetes**

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**Background and aims:** Diabetes is a major risk factor of peripheral artery disease (PAD). Measurement of ankle pressure is a simple and reliable method of assessing lower limb arterial blood supply. In diabetes, atherosclerotic regions often occur in small artery, which cannot be detected by ankle pressure measurement. Measurement of toe pressure is a method to evaluate arterial blood supply under the ankle, but it may be complicated technically, and its usefulness compared with ankle pressure has been controversial. We evaluated the usefulness of measurement of toe brachial pressure index (TBI) compared with ankle brachial pressure index (ABI) in diabetic patients with and without PAD by using a newly developed oscillometric method, that was easier than before, technically.
**Materials and methods:** TBI and ABI were measured by oscillometric methods (form PWV/ABI, COLIN medical technology Co., Ltd, Komaki, Japan) in 100 normal subjects (20–70 years old) and 135 patients with diabetes (36–70 years old). In 433 feet, 200 of normal subjects and 233 of diabetics, excluding 32 feet with high ABI (>1.29), clinical characters were investigated.
**Results:** In 200 normal feet, ABI was $1.13 \pm 0.07$, and TBI was $0.89 \pm 0.10$, both were not affected by age or gender. In 203 diabetic feet with normal ABI (>0.9), ABI was $1.12 \pm 0.07$ that was the same as in normal subjects, but TBI was $0.82 \pm 0.10$ that was lower than in normal subjects, significantly ($P<0.001$). TBI in diabetic patients with proliferative retinopathy or proteinuria was significantly lower than in diabetic patients without those complications ($0.75 \pm 0.15$ vs. $0.85 \pm 0.12$, $P=0.02$) .Other atherosclerotic disease, ischemic heart disease and brain infarction did not influence the

level of TBI. In 30 diabetic feet with PAD (ABI<0.9), TBI was significantly correlated with ABI (r=0.625, P=0.0002). Eleven feet had some severe troubles, such as ulcer, cyanosis and gangrene. Compared with 19 patients without any troubles, patients with these severe troubles were older (75 ± 14 vs. 69 ± 14 years old, P=0.03), had proteinuria (8/11 vs. 9/19) and proliferative retinopathy (8/11 vs. 12/19), and revealed lower nerve conduction velocity of median nerve (43 ± 5 vs. 52 ± 5 m/s, P=0.001). TBI in the feet with severe troubles was lower than without those features (0.36 ± 0.09 vs. 0.50 ± 0.14, P=0.007), though the levels of ABI were not differed (0.69 ± 0.14 vs. 0.75 ± 0.14).
**Conclusion:** The measurement of TBI, using a newly developed oscillometric method, is useful for detection the disorder of circulation in lower limb, even in the feet with normal ABI. In diabetic patients with advanced microangiopathy, TBI should be decreased more severely than the level of ABI. And in diabetic patients with PAD, TBI is probably more useful index for the prediction of foot troubles than ABI only.

## 1223

**Toe systolic pressure in diabetic patients with angina pectoris: a possible marker for wound complications after coronary artery bypass grafting**
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**Background and aims:** The incidence of lower extremity soft tissue wound complications after coronary artery bypass grafting (CAGB), due to vein harvest incisions has been reported to be as high as 24%. Diabetes and undetected peripheral vascular disease (PVD) are considered risk factors for these severe complications. The aim of this pilot study was to measure toe systolic pressure (TSP) and toe brachial index (TBI) in a diabetic population with angina pectoris before coronary angiograms and subsequent CABG. TSP and TBI were then compared with TSP and TBI in an age matched diabetic population. Wound complications after CABG were also registered.
**Material and methods:** 12 diabetic patients with ischemic heart disease (IHD) were examined (Age: 61 ± 11 years, males n: 11, diabetes duration: 13 ± 12 years, smokers: 58%). Brachial systolic blood pressure (BSP), diastolic blood pressure (BDP) and TSP were measured after 5 min rest. Great toe skin temperature was kept at ≥ 27°. The control population (CTR) consisted of 126 age-matched diabetic patients in two primary care centres. (Age: 66 ± 13 years ,males n: 72, diabetes duration: 8 ± 6 years, smokers: 17%). TSP was determined with a combined blood pressure cuff and pulsoximeter probe (Arterio Test MTA™ ). A pulsoximeter was used for the pulsoximeter readings (Biox-Ohmeda ™ 3800). Neuropathy was also evaluated. All measurements were done on both sides and in duplicate.
**Results:** Before the coronary angiogram BSP and BDP were 146 ± 17 and 78 ± 5 mm Hg compared with 156 ± 23 and 82 ± 10 mm Hg in the controls (p:n.s.). TSP and TBI were reduced in the IHD patients compared with CTR, TSP: 94 ± 42 versus 116 ± 34 mm Hg and TBI 0.65 ± 0.27 versus 0.76 ± 0.22, $p < 0.01$ and $p < 0.05$ repectively.25% of the IHD patients had foot ulcers and two had claudicatio intermittens. 50% of the IHD patients had neuropahty compared with 52% in the CTR. Eleven of the patients with IHD had angiograms with 2 or 3 vessel disease.One IHD patient with normal TSP and TBI had a normal angiogram.Three patients had 3 vessel disease in spite of TBI > 0.8 . Three patients had TSP ≤ 50 mm Hg in one or both legs, two of those were accepted for CABG. One underwent PCI. After CABG both patients with TSP ≤ 50 mm Hg developed limb treating wound complications due to infection and necrosis in the distal portion of the wound. One healed after 3 1/2 months and the other after 6 months.
**Conclusions:** These results indicates that diabetic patients with IHD have lower TSP and TBI than a control population with diabetes but without IHD. 25% of the IHD patients had TSP ≤ 50 mm Hg in one or both legs. After CABG these patients have an increased risk for serious wound complications at the site of the vein graft incisions. The high incidence of severely reduced blood flow and wound complications in this population recommends the use of TSP screening before CABG. Intensive foot and wound care may then be initiated to prevent wound complications. Probably could alternative surgical procedures be considered in patients with a known impairment in the peripheral circulation.

## 1224

**Elevated pregnancy-associated plasma protein-A in sera from Type 2 diabetic patients with hypercholesterolemia: associations with carotid atherosclerosis and toe-brachial index**
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**Background and aims:** We compared pregnancy-associated plasma protein-A (PAPP-A) concentrations in sera from patients with type 2 diabetes to those in sera from age-matched control subjects, and also investigated whether serum PAPP-A was associated with carotid intima-media wall thickness (IMT), an early marker of atherosclerosis, and indices of peripheral vascular disease in the diabetic patients.
**Materials and methods:** Serum PAPP-A was measured by an enzyme-linked immunosorbent assay in 103 type 2 diabetic patients and 42 age-matched control subjects. IMT was evaluated ultrasonographically in both common carotid arteries. As measures of peripheral vascular disease, we also determined the ankle-brachial index (ABI) and toe-brachial index (TBI) for systolic blood pressure. Hypercholesterolemia was defined as a serum LDL-cholesterol concentration exceeding 3.6 mmol / L, or alternatively as a treatment with hydroxymethylglutaryl coenzyme A reductase inhibitor.
**Results:** Serum PAPP-A was significantly higher in diabetic patients than in control subjects (P<0.0001). In diabetic patients, serum PAPP-A correlated positively with serum total cholesterol (r=0.289, P=0.0041) and IMT (r= 0.315, P=0.0017), and negatively with TBI (r= −0.294, P=0.0039), but not ABI. Diabetic patients with hypercholesterolemia had higher PAPP-A concentrations than those without hypercholesterolemia [median (interquartile ranges; 8.37 (6.93, 11.6) vs. 7.29 (5.65, 9.21) mIU/L; P=0.0209)]. Multivariate analysis identified only serum total cholesterol as an independent determinant of serum PAPP-A in patients with type 2 diabetes (partial coefficient= 0.454, P=0.020).
**Conclusion:** Serum PAPP-A concentrations were significantly elevated in diabetic patients with hypercholesterolemia, and were associated positively with carotid atherosclerosis and negatively with TBI in type 2 diabetes.

## 1225

**Diabetic macroangiopathy feature in patients with diabetes mellitus Type 2 and arterial hypertension**
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**Backgrounds and aims:** Diabetic macroangiopathy (DMA) of lower extremities is characterised by a gradual reduction in blood flow to one or more limbs secondary to atherosclerosis. The prevalence of DMA is 2 – 6% for men and women younger than 50 years old, increasing to more than 7% in those older than 70 years old. Hyperglicemia, smoking, hypertension and hyperlipidemia are important risk factors for DMA. The aim was to expose clinical and functional diabetic macroangiopathy feature in type 2 diabetes (DM 2) patients with arterial hypertension.
**Materials and methods:** 70 patients with DM 2 and arterial hypertension were studied. Ultrasound high resolution B-mode imaging of the main leg arteries was conducted. The intima-media thickness (IMT) of common femoral arteries was measured 2 cm proximal to bifurcation and the presence or absence of plague on main leg arteries was recorded. Flow-mediated vasodilatation (FMD) of brachial artery was examined 60 seconds after release of the occlusive cuff.
**Results:** Only 48 (68.57%) of the patients had the symptoms of intermittent claudication. Most of the patients had excessive weight (body mass index > 25 kg/m$^2$) and a not satisfactory diabetic control (HbA1 c −8.3 ± 1.16%). Exclusion criteria was significant retinopathy and nephropathy. 93% of the patients had diabetic neuropathy. According to the results of ultrasound high resolution B-mode imaging of main leg arteries, all the patients were divided into 3 groups: group 1 (20 patients) had at least one occluded or stenosis artery in the femoral-popliteal segment, group 2 (22 patients) - in the arterial segment below the knee, group 3 (28 patients) did not detect any hemodynamically significant changes. All patients were comparable by age, sex, duration of DM and body mass index, level of systolic blood pressure. The duration of hypertension was higher in group 1, and in some cases hypertension earlier than DM.
All the patients had the increased IMT of common femoral artery; it was more significant in group 1 compared to groups 2 and 3 (1.54 ± 0.49 vs. 0.94 ± 0.23 and 0.83 ± 0.24 mm). FMD of brachial artery was conducted to the

patients of study group and to 10 subjects with hypertension, without DM (control group). Assessment of FMD revealed that the patients of group 1 had the worst vasodilatation.

| | Group 1 | Group 2 | Group 3 | Control group |
|---|---|---|---|---|
| Baseline diameter M ± m, mm | 3.77 ± 0.29 | 4.1 ± 0.31 | 3.89 ± 0.25 | 3.78 ± 0.26 |
| Velocity of blood flow, baseline, sm/s | 83.7 ± 10.2 | 76.5 ± 12.1 | 94.1 ± 14.8 | 89.3 ± 10.23 |
| FMD, % | 4.2 ± 0.4* | 4.7 ± 0.7* | 6.7 ± 0.9 | 9.5 ± 0.5* |
| Increase in velocity of blood flow, % | 168.7 ± 19.4 | 153.3 ± 15.6 | 172.7 ± 21.3 | 163.7 ± 19.5 |

*- $p < 0,05$

We observed no correlation between IMT of common femoral artery and sex, age, glycosylated hemoglobin A1 c level, serum triglycerides, systolic blood pressure.
**Conclusion:** Hypertension is the primary risk factor of peripheral artery disease in femoral-popliteal segment and hyperglycemia promotes atherosclerosis in the arterial segment below the knee.

## 1226

**Postoperative glucose levels are an independent risk factor for infections after peripheral vascular surgery**

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**Background and aims:** An independent relationship between postoperative hyperglycaemia and occurrence of postoperative infections has been described in patients undergoing coronary artery surgery. We evaluated whether hyperglycaemia in the first 48 hours after infrainguinal vascular surgery is a risk factor for postoperative infections, independent from factors associated with insulin resistance and surgical stress.
**Patients and methods:** Patients who underwent infrainguinal vascular surgery in our hospital between 1-3-1998 and 1-3-2003 were included. Glucose values were retrieved from laboratory reports until 48 hours after surgery and were averaged per patient. Postoperative infections were scored during hospital stay until a maximum of 30 days after surgery and only when treated with antibiotics. Data were analysed with univariate and multivariate logistic regression analysis.
**Results:** In total 275 patients were included, 15 (5.5%) of whom had previously diagnosed type 1 diabetes and 55 (20%) previously diagnosed type 2 diabetes. The incidence of postoperative infections was 84/275 (31%). At least one postoperative glucose value could be retrieved in 211/275 (77%) patients (median 3 values per patient). The median of averaged postoperative glucose values was 6.5 mmol/l (interquartile range: 5.7–8.4 mmol/l). Factors associated with postoperative infections, besides postoperative glucose levels, were: sex, age, body mass index, diabetes mellitus, Fontaine classification, American Society of Anesthesiologists classification, duration of surgery and the surgeons' rank. When corrected for these factors, postoperative glucose levels were found to be an independent risk factor for postoperative infections (odds ratio fourth quartile versus first quartile: 5.1; 95% confidence interval: 1.6–17.1; p= 0.007).
**Conclusion:** Postoperative infections occur in almost one-third of all patients undergoing infrainguinal vascular surgery. Our data suggest that postoperative glucose levels are an independent risk factor for infections after infrainguinal vascular surgery. After confirmation in a prospective cohort study, the effect of strict glucose control in patients undergoing infrainguinal vascular surgery should be investigated in a randomised clinical trial.

# PS 127
# Intima media thickness

## 1227

**Isolated impaired glucose tolerance but not isolated impaired fasting glucose is associated with rapid progression of intima media thickness – a 3 year-follow-up observation of the RIAD-study**

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**Background and aims:** Impaired glucose tolerance (IGT) but not isolated fasting glucose (IFG) has been found to be associated with increased intima media thickness of common carotid arteries (CCA) despite a similar level of major risk factors. Only scarce information exists about progression of IMT in different types of prediabetic glucose tolerance.
**Material and methods:** A total of 352 middle-aged subjects participate on the Risk Factors in Impaired Glucose Tolerance for Atherosclerosis and Diabetes (RIAD) underwent a 75-g oral glucose tolerance and were classified as follows: 164 with normal glucose metabolism (NGT), 49 with impaired fasting glucose (IFG), 41 impaired glucose tolerance (IGT) and with 53 combined hyperglycaemia (IFG/IGT) and 45 with newly diagnosed diabetes mellitus respectively. IMT was measured by high resolution ultrasound at baseline and after 3-years.
**Results:** No significant changes of IMT was seen in NGT (0,83 vs 0,82 mm; n.s.) and IFH (0,85 vs 0,80 mm; n.s.). A significant increase of IMT was seen in IPH (0,81 vs. 0,89 mm; p=0,016), FH/PH (0,86 vs. 0,94 mm; p<0,001) and diabetes (0,83 vs. 0,93 mm; p<0,001).
**Conclusion:** IPH, FH/PH and diabetes are associated with accelerated atherosclerosis of common carotid arteries over a period of 3 years compared with NGT. Isolated impaired fasting glucose was not a risk factor for carotid atherosclerosis.

## 1228

**Absolute risk of cardiovascular disease and carotid atherosclerosis in diabetic patients**

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**Background and aims:** The UKPDS-Risk Engine (www.dtu.ox.ac.uk/) represents a handy tool for the evaluation of the absolute risk of coronary heart disease (CHD)/stroke in type-2 diabetic patients. Aim of the study was to correlate the carotid atherosclerosis, evaluated by the Intima-Media-Thickness (IMT) of these arteries, with the absolute risk of CHD/stroke estimated by the UKPDS-Risk Engine .
**Material and methods:** 200 diabetic outpatients (M:96, F:104) with no evidence of cardiovascular disease were included. Variables used were: age, sex and the known risk factors smoking, diabetes duration, systolic blood pressure, atrial fibrillation, total and HDL cholesterol and glucosylated haemoglobin. The IMTs of both Internal (ICA) and Common Carotid arteries (CCA) measured by B-mode U/S were correlated with the absolute risk of CHD/stroke. Patients were also matched into 3 groups according to their level of absolute risk of CHD/stroke for the following 10 years (<10%, 10–20%, >20%) and the mean IMTs of these 3 groups were compared. Results were statistically evaluated by Pearson's correlation coefficient and the ANOVA technique.
**Results:** Table 1 shows the correlation between the estimated absolute risk of cardiovascular disease and the carotid IMT, while Tables 2 and 3 depict the 3 risk groups with their corresponding IMTs.

| Carotid - IMT | CHD | | STROKE | |
|---|---|---|---|---|
| | r | p | r' | p' |
| ICA | 0.328 | 0.001 | 0.236 | 0.019 |
| CCA | 0.386 | 0.000 | 0.234 | 0.020 |

TABLE 1. CORRELATION OF ABSOLUTE RISK AND IMT

| Level | N (%) | IMT-ICA (mean ± SD) | IMT-CCA (mean ± SD) |
|---|---|---|---|
| <10 % | 16 (8) | 0.557 ± 0.132 | 0.639 ± 0.283 |
| 10–20 % | 30 (15) | 0.762 ± 0.251 | 0.712 ± 0.143 |
| >20 % | 154 (77) | 0.928 ± 0.363 | 0.911 ± 0.354 |
| *TOTAL* | 200 (100) | 0.876 ± 0.352** | 0.862 ± 0.337* |

(*) p<0.05 , (**) p< 0.01 (ANOVA between groups)
TABLE 2. LEVEL OF CHD - RISK AND CAROTID - IMT

| Level | N(%) | IMT-ICA (mean ± SD) | IMT-CCA (mean ± SD) |
|---|---|---|---|
| <10 % | 60 (30) | 0.706 ± 0.234 | 0.713 ± 0.188 |
| 10–20 % | 62 (31) | 0.891 ± 0.365 | 0.856 ± 0.250 |
| >20 % | 78 (39) | 1.000 ± 0.371 | 0.986 ± 0.436 |
| *TOTAL* | 200 (100) | 0.877 ± 0.352* | 0.862 ± 0.338* |

(*) p< 0.01 (ANOVA between groups)
TABLE 3. LEVEL OF STROKE - RISK AND CAROTID - IMT

**Conclusion:** The carotid IMT was positively correlated with the absolute risk of CHD/stroke estimated by the UKPDS – Risk Engine. Increasing levels of risk (<10%, 10–20%, >20%) were also associated with statistically significant increase of the carotid IMT.

## 1229

**Endothelial function and carotid intima media thickness in adult Type 1 diabetics-relationship to apoE polymorphism and cardiovascular risk factors**

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**Background and aims:** Several studies could demonstrate an impaired endothelial function (ED) and an increased carotid intima media thickness (IMT) in type 1 diabetics compared to the age-matched non-diabetic population.
The present evaluation was performed to study differences in the relationship of IMT and ED to apoE polymorphism, classical cardiovascular risk factors and markers of subclinical inflammation.
**Materials and methods:** Clinical and laboratory data of 44 type 1 diabetics (15 women, 29 men; mean age 30.5+/–6.2 years; mean duration of diabetes 11.2+/–8.3 years) treated by a basis-bolus insulin regimen, were evaluated. None of the patients showed clinical signs of manifest cardiovascular disease, microalbuminuria was positive in 18 patients. These patients were treated by ACE-inhibitors. Laboratory results and ultrasound controls were performed in the morning after an overnight fasting period and after administration of the basal insulin dosage.
Results are expressed by means+/–SD, comparison between the groups were conducted by the Student`s t test. Associations were analyzed by calculating the Pearson`s and Spearman`s corrrelation coefficients.
**Results:** Mean IMT was greater in men (0.59+/–0.10 mm) than in women (0.50 +/–0.10 mm; p < 0.002), and greater in apoE4 / E2 phenotype (0.58 + / – 0.10 mm) than in apoE3/E3 (0.51+/–0.10 mm;p<0.001). Mean carotid IMT revealed a significant positive correlation with age (R=0.36;p<0.019), body mass index (R=0.34;p<0.032), fasting glucose (R=0.38;p<0.015), diastolic blood pressure (R = 0.39; p < 0.012), plasma ferritin (R = 0.36; p<0.021) and homocysteine values (R=0.34;p<0.032). Flow mediated vasodilatation (FMD) was greater in women (5.58+/–6.00%) compared to men (3.55 + / – 3.60%). FMD only correlated with the presence of microalbuminuria (R=0.35; p<0.021) and the plasma levels of tumor necrosis factor (TNF)-alpha in men (R=0.58;p<0.001), but with no other risk factors. The positive relationship of FMD to microalbuminuria might be explained by the ACE-inhibitor therapy in these patients.
**Conclusion:** The impaired ED is known to precede an increase in IMT. The results of our evaluation indicate, that only IMT values show a positive correlation to the cardiovascular risk factor profile in adult type 1 diabetics, and might thus be more appropriate to control the vascular effects of risk factor management under clinical routine procedures.

## 1230

**In Type 2 diabetes mellitus metabolic control does correlate with calcific rather than early atherosclerotic involvement.**

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**Background and aims:** Intima-Media Thickness (IMT) is an index of early atherosclerosis (ATS) while calcific plaques represent a marker of advanced disease.
**Materials and methods:** In each of 137 type 2 diabetic patients (61 males, 76 females, mean age 61 ± 9.7 yrs, mean diabetes duration, DD, 12.5 ± 10.2 yrs) were checked: blood pressure (BP), smoking habits, BMI, total-, HDL-, LDL-cholesterol (Cho); plasma uric acid (pUA), fibrinogen, C-reactive protein (CRP), tHcy, urinary-albumin excretion rate (UAER), fasting plasma glucose (FPG), fructosamine (FR), glycosylated hemoglobin (GHb), mean pre- and post-prandial glycemic sticks (MPPsticks), Glomerular Filtration Rate (GFR) together with retinopathy and neuropathy. Carotid vessel B-mode ultrasonography was accomplished and IMT was taken on-line bilaterally on frozen images of the distal common carotid posterior wall; right + left measurements were averaged to give mean-IMT (m-IMT). To search vascular calcific plaques, aortic and peripheral vessel xrays were performed. Twenty healthy sex-age-matched subjects served as controls.
**Results:** In diabetics, the mean IMT was 0.926 ± 0.231 mm, in controls 0.552 ± 0.021 mm. Mean-IMT correlated with patients' age (p <.0001), systolic BP (p.02), pulse pressure (p.01), total- (p.009) and LDL-Cho (p.03), pUA (p.01), tHcy (0.04) as also with the presence of aortic and peripheral atherosclerosis, not with the metabolic control or microvascular involvement. An internal score including the presence or not of ultrasonographic carotid plaques, aortic and/or peripheral vessel calcific plaques xray-detected significantly correlated with patients' age (p<.0001), DD (p.0008), systolic BP (p.002), pulse pressure (p<.0001), LDL-Cho (p.04), GFR (p.003), UAER (p.006), fibrinogen (p.007), CRP (p.05) not only, but even with FPG (p.004), FR (p.01), GHb (p.02), MPPsticks (p.0004) as well with the presence of diabetic retinopathy and neuropathy.
**Conclusion:**In type 2 diabetics the mean-IMT is higher than in controls and significantly related to classic cardiovascular risk factors (age, arterial hypertension, lipid disorders), not with metabolic control. Only widespread, advanced atherosclerosis, ultrasonography or xray detected, strongly correlates with the metabolic control as well as with the macro and microvascular involvement, in addition to classic cardiovascular risk indicators.

## 1231

**Predictors for carotid plaque formation as a marker of the early progression of carotid atherosclerosis in Type 2 diabetes**

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**Background and aims:** Newly plaque formation of carotid artery may represent early atherosclerosis. Although several studies have evaluated risk factors for increasing carotid intima-media thickness (IMT), few information are available concerning risk factors for the carotid plaque formation in type 2 diabetes. The aim of this study was to determine the predictors for carotid plaque formation in type 2 diabetes.
**Materials and methods:** We studied, prospectively for 1 year, 75 type 2 diabetic outpatients of 40 to 84 years of age (44 females and 31 males, mean age; 61.4 years), who have the possibility of carotid plaque formation from preliminary study in type 2 diabetes. These patients were confirmed the absent of carotid plaque formation at baseline by ultrasonographic assessment, and bilateral carotid IMT and aortic pulse wave conduction velocity (PWV) were measured. At over night fast, blood pressure (BP) was measured, and blood sampling for plasma glucose, HbA1 c, serum lipids and lipoprotein (a)[Lp(a)] were performed. Urinary albumin excretion ratio (AER) was determined in morning spot urine sample. One-year later, ultrasonographic assessment, including carotid plaque formation, was reevaluated; plaque score (PS), a maker of the severity of carotid atherosclerosis, was obtained by summing up the maximum thickness of all plaques in bilateral carotid arteries. Patients were divided into two subgroups with or without carotid plaque.
**Results:** Newly carotid plaques were found in 53 patients (71%), number of carotid plaques was 2.2+/–1.2 (mean+/–SD) and PS was 3.1+/–1.8 mm, respectively. No differences between two groups were observed in age, gender and duration of diabetes. Systolic BP in patients with carotid plaque was significantly higher than that in patients without carotid plaque (140+/–18 vs. 130+/–12 mmHg, P < 0.01) at baseline, but diastolic BP was

not different. Furthermore, log Lp(a) in patients with carotid plaque was greater than that of in patients without carotid plaque (1.23+/–0.30 vs. 1.04+/–0.38, P<0.02), and initial log Lp(a) in all patients were positively correlated with PS (r=0.321, P<0.01). Moreover, increased bilateral IMT values were observed in patients with carotid plaques compared to the patients without carotid plaques (left: 0.68+/–0.10 vs. 0.61+/–0.07 mm, P<0.01, and right: 0.65+/–0.09 vs. 0.60+/–0.06 mm, P<0.05, respectively) at baseline. During the follow up period, IMT in patients without carotid plaque was showed no significant changes. However, bilateral IMT in subjects with carotid plaque during the period were significantly increased (left: 0.68+/–0.10 to 0.77+/– 0.12 mm, P<0.01, and right: 0.65+/–0.09 to 0.72+/–0.12 mm, P<0.01, respectively). PWV and log AER in patients with carotid plaque were also greater compared with those in patients without carotid plaque at baseline. Stepwise multivariate analysis using initial values demonstrated that independent predictive values for carotid PS were log Lp(a) (F=7.73, P=0.007) and right-IMT (F=6.73, P=0.011), respectively.
**Conclusion:** These results suggest that initial serum Lp(a) levels and right-IMT are diagnostic predictors for carotid plaque formation, and are associated simultaneously with the degree of early carotid atherosclerosis in type 2 diabetes.

## 1232

**Association of metabolic syndrome components with carotid intima-media thickness in Korea**

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**Background:** The measurement of carotid artery intima-media thickness (IMT) by high-resolution B-mode ultrasonography has been proven to be useful, repeatable, and reliable method of detecting subclinical atherosclerosis. Thus, the aim of our study is to investigate the association of cardiovascular risk factors with carotid IMT of type 2 diabetic patients in Korea.
**Method:** A total of 535 patients with type 2 diabetes were recruited and their IMTs on common carotid artery (CCA) and risk factors of atheosclerosis such as anthropometric and biochemical profiles were examined. Insulin resistance was measured by the short insulin (Humulin-R, Eli Lilly, U.S.) tolerance test. The insulin sensitivity index (ISI, %/min) was calculated by linear regression from the rate of the fall of the log value of glucose between 3 and 15 min.
**Result:** The male subjects have thicker IMT than female subjects (0.88 ± 0.26 mm v.s. 0.80 ± 0.21 mm, $p < 0.05$). Among the metabolic variables, IMT was significantly correlated with age (r=0.378, p=0.001), smoking (r=0.253, p=0.034), systolic blood pressure (r=0.097, p=0.049), body mass index (BMI) (r = 0.083, p = 0.025), waist hip ratio (WHR) (r = 0.116, p = 0.002), LDL-cholesterol (r=0.089, p=0.040), HDL-cholesterol (r= –0.106, p = 0.030), fasting C-peptice (r = 0.310, p = 0.001) and ISI (r = –0.297, p = 0.015). However, the correlation of IMT with LP-(a) or fibrinogen was not significant. Also, carotid IMT increases with the numbers of several risk factors.
**Conclusion:** These results suggested that cardiovascular risk factors including insulin resistance were involved each other in subclinical atherosclerosis of patients with metabolic syndrome.

# PS 128
# Stroke

## 1233

**The investigation of cerebral blood flow and reserve capacity in diabetes mellitus**

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**Background and aims:** The occurrence of myocardial infarction and stroke is 3–4 times higher in Type 2 diabetics when compared to the healthy population. Macroangiopathy is less common in Type 1 diabetes. Carotid artery ultrasound examination is a wildly used and accepted method to assess the velocity of blood flow both in the carotid artery and Willis-ring, but it gives only little information about the amount of the blood flowing through the brain and the reserve capacity of the cerebral arteries. The functional capacity of the brain arteries of Type 1 and Type 2 diabetic patients without any signs of central nervous system involvment was examined with Tc99 mHMPAO SPECT (technetium-99 m hexametil propilénamin oxim - single photon emission computer tomography). Results of the two diabetic groups were compared and possible association with various vascular haematological parameters like CRP, fibrinogen and homocystein level was investigated.
**Materials and methods:** 64 diabetic patients were involved in the study (18 Type 1: age: 34.29 ± 10.08 years, duration of diabetes: 10.00 ± 10.17 years and 46 Type 2: age: 54.89 ± 7.81 years, duration of diabetes: 6.89 ± 5.82 years). Cerebral blood flow was examined by a two day protocol. Basal and subsequently Diamox-stimulated cerebral blood flow was measured on two different occasions. Diamox was given 15 minutes before the injection of the radiopharmacon and reserve capacity was calculated on the basis of the difference between stimulated and basal blood flow. The activity of the radiopharmacon was 750 MBq on both occasion. Blood flow was given as ml/min/100 g brain tissue. The flow of the hemisphaers were separately given. CRP, fibrinogen and homocystein level were also measured. Statistical analysis was performed by Student's t-test.
**Results:** both basal and the Diamox stimulated cerebral blood flow was lower in Type 2 diabetics (Type 1 diabetes: resting flow: right hemisphaer: 56.40 ± 7.66, left hemisphaer: 56.26 ± 10.07, Diamox stimulated flow: right hemisphaer: 64.25 ± 10.36, left hemisphaer: 61.50 ± 10.45; Type 2 diabetes: resting flow: right hemisphaer: 49.21 ± 6.15, left hemisphaer: 47.78 ± 6.30, Diamox stimulated flow: right hemisphaer: 53.48 ± 7.45, left hemisphaer: 52.04 ± 7.90, $p<0.05$), the reserve capacity, however, was the same in both groups. Cerebral blood flow was declining by age. After correcting for this age-dependent phenomenon cerebral blood flow of Type 2 diabetic patients still remained lower when compered to that of Type 1 diabetics, although the difference was not significant. In Type 2 diabetics significantly higher CRP, fibrinogen and homocystein level was measured in comparison to the Type 1 group.
**Conclusion:** Decreased cerebral blood flow of Type 2 diabetic patients compared to Type 1 diabetics might represent a more pronounced macrovascular injury and significantly elevated CRP, fibrinogen and homocystein levels seem to have a possible role in the pathogenesis.

## 1234

**Interleukin-1 receptor antagonist polymorphism is not associated with ischemic stroke in Type 2 diabetes**

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**Background and aims:** The interleukin-1 receptor antagonist (IL-1ra) is a major modulator of the interleukin-1 pro-inflammatory pathway. Recently, IL-1ra polymorphism has been shown to be associated with type 2 diabetes and ischemic stroke. We studied the relationship between a variable number tandem repeat (VNTR) polymorphism in intron 2 of the IL-1ra gene (IL1RN) and ischemic stroke in patients with and without type 2 diabetes.
**Materials and methods:** Two hundred seventy two ischemic stroke patients (123 with type 2 diabetes), 373 healthy matched control subjects and 157 type 2 diabetic patients without ischemic stroke (diabetes duration ≥ 10 years) were genotyped for IL-1ra polymorphism by polymerase chain reaction (PCR).
**Results:** IL1RN*1/IL1RN*2 genotype and IL1RN*2 allele frequency were higher in ischemic stroke patients than those observed in the control group

(10.3 vs. 4.0%, OR=2.74, P=0.0016; and 5.5 vs. 2.0%, OR=2.84, P=0.0007; respectively). Genotypic analysis revealed that type 2 diabetes patients shown a higher prevalence of the IL1RN*1/IL1RN*2 genotype (10.7%) than controls (4.0%)(OR=2.86, P=0.0008). And ischemic stroke patients with type 2 diabetes displayed a greater prevalence of the IL1RN*1 / IL1RN*2 genotype (13.8%) than controls (4.0%)(OR=3.83, P=0.0001). However, IL1RN*1/IL1RN*2 genotype and IL1RN*2 allele frequency of type 2 diabetes patients with ischemic stroke were not different from those observed in the type 2 diabetes patients without ischemic stroke.
**Conclusion:** These results suggest that the IL1RN*2 allele of IL-1ra polymorphism may be associated with susceptibility for ischemic stroke and type 2 diabetes, and it may play as an accelerating factor, but not a predictive marker for ischemic stroke in type 2 diabetes.
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## 1235

**Association of lacunar infarction with carotid intimal-media wall thickness in patients with Type 2 diabetes**
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**Background and aims:** Diabetes is a well-established risk factor for lacunar infarction (LAC) and atherosclerotic carotid artery wall thickening. To our knowledge, however, an association between LAC and carotid intimal-media wall thickness (IMT) has not been clearly demonstrated. The present study was therefore undertaken to compare the risk factors for LAC and IMT and to investigate the association between LAC and IMT in patients with type 2 diabetes.
**Materials and methods:** The subjects of this study were 34 type 2 diabetic patients with no history of stroke (age 64.1 ± 10.3 years, 23 males and 11 females). LAC was diagnosed based on the presence of hyperintensity on T2-weighted MRI images, and the IMT of the common carotid artery was measured by ultrasonography. We examined the following risk factors for LAC and IMT: serum HbA1 c, lipid and creatinine (S-Cr) levels, age, sex, smoking habit, blood pressure (BP), and urinary albumin excretion (UAE).
**Results:**The 17 patients with LAC (68.2 ± 9.4 years) were significantly older ($p<0.01$) than the 11 patients without LAC (59.8 ± 9.6 years), but there were no significant differences between the two groups in regard to BP, serum HbA1 c, lipid and S-Cr levels, or UAE. On the other hand, 22 patients showed increased IMT defined as >1.0 mm, and male and smoking habit were significantly higher ($p<0.01$) in the patients with increased IMT than those in the patients with normal IMT. The rate of LAC in the patients with increased IMT (69.0%) was significantly higher ($P<0.001$) than in the patients with normal IMT (36.3%). IMT was positively correlated with S-Cr levels ($r=0.566$, $p<0.01$). Next, we divided the patients into two groups according to their age. In the 21 patients under 70 years of age, the patients with increased IMT showed a pronounced increase in UAE compared with the patients with normal IMT (302.9 ± 358.2 mg/gCr vs 63.4 ± 80.9 mg/gCr, $p<0.05$). The 10 patients with both LAC and increased IMT were significantly older than the 7 patients with neither LAC or increased IMT (72.8 ± 5.2 years vs 59.7 ± 7.9, $P<0.001$), but there were no significant differences in BP, serum HbA1 c, lipid and S-Cr levels, or UAE between the two groups.
**Conclusion:** Carotid artery wall thickening was linked to lacunar infarction. Aging was shown to be a risk factor for both lacunar infarction and carotid artery wall thickening, and male, smoking habit, urinary albumin excretion and renal function were important risk factors for carotid artery wall thickening.

## 1236

**Incidence of diabetes and impaired glucose metabolism in subjects with the ischaemic stroke**
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**Introduction:** Incidence of diabetes and impaired glucose metabolism in patients with a stroke is still unknown, because hyperglycaemia observed very often during acute phase of stroke is difficult for interpretation and usually is considered to be a stress hyperglycaemia.
**Aim of study:** To assess incidence of diabetes and other forms of impaired glucose metabolism in subjects with the ischemic stroke.
**Material and methods:** Study was conducted in 220 patients of age 23–94 years (mean 67.2 y), 96 women and 124 men. Patients were included within acute phase of ischemic stroke, after CT examination. Performed measurements: admission plasma glucose (venous plasma level), diurnal glycaemia (whole capillary blood) measured for 3–14 days, concentration of glycated haemoglobin (HbA1 c).
**Results:** In 43 of 220 investigated subjects (19,5%) diabetes was diagnosed before the stroke. In 20 subjects (9,5%) diabetes was diagnosed during acute phase of stroke – criteria: glucose level ≥200 mg/dl (>11 mmol / l) in diurnal glycaemia and elevated HbA1. Unclassified hyperglycaemia – fasting glucose level >5,5 mmol / l (≥100 mg / dl) or diurnal glucose level ≥ 7,8 mmol / l (≥140 mg/dl) with normal HbA1 c was found in 155 of patients (70%). Normal glucose levels were observed only in 2 subjects (1%). After 6 month period oral glucose tolerance test was performed in 55 of 155 subjects with unclassified hyperglycaemia. Diabetes was diagnosed in 18 patients (33 %), impaired glucose tolerance found in 12 subjects (22%), impaired fasting glycaemia occured in 8 subjects (14 %). Only 17 patients (31%) appeared to be euglycaemic.
**Conclusions:** 1. Diabetes mellitus can be found in 1/3 of patients with acute phase of ischaemic stroke and its presence can be assumed in more than half of them. 2. Impaired glucose tolerance and impaired fasting glycaemia are present in 1/3 of patients with unclassified hyperglycaemia observed during acute phase of stroke 3. Only 1/3 of hyperglycaemia found in acute phase of ischaemic stroke occurs to be stress hyperglycaemia.

## 1237

**Epidemiology of stroke in a cohort of Type 2 diabetics – 10-year follow-up**
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**Background and aims:** Stroke is one of the main causes of death and disability in type 2 diabetics. The study was aimed at the analysis of prevalence and incidence of stroke in a cohort of 1334 type 2 diabetic persons during 10-year follow-up. The study had to point to the factors promoting a development of stroke.
**Materials and methods:** All studied patients have attended an out-patient diabetological clinic since 1992 or longer. The diagnosis of stroke was established on the base of focal central nervous system disturbances lasting more than 24 hours. We analyzed an impact of age, sex, diabetes duration, BMI, lipid profile, fasting and postprandial glycemia, 24-hour proteinuria, serum creatinine, smoking, hypertension and other vascular complications on stroke.
**Results:** At baseline previous stroke was found in 62 (4.6%) persons – 34 females and 28 males. In years 1992 – 2001, 135 episodes of stroke were observed. We analyzed these cases of stroke which appeared for the first time in the life. During 10-year follow-up among 1272 persons, who had not had stroke before 1992 we found new episodes of stroke in 100 patients. In this group 15 persons experienced 2 stroke episodes and one – 3 stroke episodes. Cumulative incidence of stroke in the study cohort was 12.1%. In 44 cases the first stroke was the cause of death. Mean age at the moment of diagnosis of the first stroke was 69.8 ± 9.8 years and mean diabetes duration was 13.4 ± 6.6 years. Incidence of stroke in this group was 10.8 per 1000 person-years. In a group of 62 persons after stroke before 1992 next episodes of stroke appeared in 16 subjects. In 8 cases stroke was the cause of death. Patients who experienced stroke during the follow-up were older, more obese, had higher glycemia and higher daily proteinuria in comparison with the patients without stroke. The 10-year survival of the group free of stroke at baseline was significantly lower than in the group after stroke – 65.6% vs 33.9% ($p<0.001$). In the whole cohort stroke was the cause of death in 11.0% of persons. It was the third cause of death (after ischemic heart disease and neoplasms).
**Conclusion:** The epidemiological indices of stroke in the cohort under study are very high. This study gives us much information which may be helpful in planning health care and treatment strategies in type 2 diabetic patients.

# PS 129

# Endothelial dysfunction and adiponectin

## 1238

**The effect of two consecutive fat-rich mixed-meals on endothelial function and cell-derived microparticles in healthy well-trained subjects**

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**Background and aims:** Clinical and epidemiological evidence indicates that postprandial elevations of plasma glucose and triglycerides are related to the risk of cardiovascular disease (CVD). Endothelial dysfunction is important in the pathogenesis of CVD and endothelium-dependent vasodilation is decreased in the postprandial state. Even in healthy subjects a single fat- or glucose load results in metabolic changes and endothelial dysfunction. Upon activation or during apoptosis several blood cells release small vesicles or microparticles (MP). Elevated numbers of MP circulate in patients at risk of thromboembolic events and CVD. However, the influence of postprandial metabolic changes on microparticle formation, in association with endothelial function, is unknown. The aim of this study was to assess the cumulative effect of two consecutive fat-rich mixed meals, consumed as breakfast and lunch, on endothelial function, measured as flow mediated dilation (FMD) by ultrasound, and the formation of cell-derived MP.
**Methods and materials:** Seventeen healthy insulin-sensitive ($HOMA_{IR}$ = 0.98 (SD 0.32)) athletic male subjects were studied during 2 visits, in random order. At visit A, subjects were given two standardised fat-rich mixed-meals (50g fat, 55g carbohydrate, 30g protein) at the start (breakfast) and at 4 h (lunch). During visit B, subjects remained fasted for 10 h. Blood samples were collected before and at 2, 4, 6 and 8 h following the first meal, to assess changes in plasma glucose, insulin, lipids and MP. Prior to each blood collection, FMD of the brachial artery was measured. MP were isolated and characterised with respect to their numbers and cellular origin by flow-cytometry.
**Results:** During visit A, plasma glucose, triglyceride and insulin levels increased significantly, especially after the second meal, as compared to baseline and visit B (both $p<0.05$, $p<0.01$, $p<0.01$, respectively). Mean plasma glucose levels rose from 4.8 (0.3) to 5.4 (0.4) mmol/l and triglycerides increased from 0.8 (0.2) to 1.7 (0.7) mmol/l (t=6 h). In addition, following the second meal, FMD was significantly impaired (6.9 % vs 3.7%, $p<0.05$). Finally, a marked increase in total numbers of MP was observed during visit A, whereas no changes were found during control visit B ($p<0.05$).
**Conclusions:** Even in healthy, insulin sensitive males, exposure to two consecutive fat-rich mixed meals results in a cumulative triglyceridaemic response, and in a concomitant gradual impairment of FMD. These adverse postprandial metabolic and vascular changes were accompanied by increased numbers of cell-derived MP. These findings may have clinical consequences for patients with prolonged postprandial metabolic abnormalities, as for example persons with the metabolic syndrome and / or type 2 diabetes mellitus.

## 1239

**High fasting plasma glucose is associated with impairment of endothelial function in insulin-resistant non-diabetic Brazilian subjects.**

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**Background and aims:** Insulin-resistance (IR) is an independent risk factor for atherosclerosis and cardiovascular mortality, even in normotolerant subjects. This fact could be explained by the impairment of endothelial function. The aim of this study was to observe differences in vascular reactivity in IR subjects enrolled by the ATPIII criteria.
**Materials and methods:** We studied 52 normotolerants IR subjects (NMT – 39 women, 13 men), aged 38.8 ± 8.37 years, BMI 36.2 ± 5.21 Kg/m$^2$; 11 glucose intolerant IR subjects (IGT – 8 women, 3 men), aged 42.0 ± 7.15 years, BMI 39.5 ± 6.56 Kg / m$^2$ and 9 control subjects (C – 4 women, 5 men), aged 26.1 ± 4,42 years, BMI 21.7 ± 1.71 Kg / m$^2$. Blood samples were collected after 12 h fasting for glucose (FPG), insulin, lipid profile, C-reactive protein (CRP), fibrinogen and 2 h post 75g glucose load. Forearm blood flow (FBF) was measured with strain gauge venous occlusion plethysmography after increasing doses of acetylcholine (Ach – 7.5, 15 e 30 mcg / min) and sodium nitroprusside (SNP – 2, 4 e 8 mcg / min) to assess endothelium dependent and independent vasodilation, respectively. Meanwhile blood pressure was measured to assess vascular resistance at each dose.
**Results:** IGT subjects had higher systolic BP (155.0 ± 16.0 mmHg vs 140.7 ± 17.7, $p < 0.05$), FPG (108 [85–120] vs 88 [85–120] mg /dL, $p < 0.05$) and posprandial PG (157 [145–198] vs.107.5 [62–125] mg /dL, $p < 0.01$) than NMT subjects. IGT group had lower variation on vascular resistance after the higher Ach dose than NMT group (–39 [–80 – 33] vs –75 [–90,4 – 29]%, $p < 0.01$). There were no differences between groups regarding blood flow responses after Ach and SNP. Linear regression showed that FPG was the only variable correlated with a lower response on vascular resistance due to endothelial-dependent vasodilation ($r = 0.29$, $p = 0.01$).
**Conclusion:** Higher levels of FPG, even at normal range in IR Brazilian subjects, were associated with impairment of vascular reactivity.
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## 1240

**Factors associated with pulse pressure in middle-aged Type 1 diabetic patients (the FinnDiane Study)**

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**Background and aims:** Pulse pressure is a surrogate measure of arterial stiffness and is associated with elevated mortality and cardiovascular morbidity in type 1 diabetic patients as well as in the general population. Type 1 diabetic patients have increased arterial stiffness and higher pulse pressure compared with the background population. We studied factors associated with pulse pressure in middle-aged type 1 diabetic patients.
**Materials and methods:** A cross-sectional analysis of 947 type 1 diabetic patients (55% males) aged 40 to 60 years participating in the ongoing, nation-wide, multi-centre FinnDiane study was performed. Blood pressure was measured twice with a mercury sphygmomanometer on a single occasion. The urinary albumin excretion rate (AER) was calculated as the mean of three urine collections. Glomerular filtration rate (GFR) was calculated using the Cockroft-Gault formula. Values are presented as mean ± SD or median [range].
**Results:** The variables age (48.8 ± 5.1 years), duration of diabetes (27.5 ± 11.3 years), sex, BMI (25.7 ± 3.5 kg/m$^2$), waist-hip ratio (0.89 ± 0.09), current smoking (0 [0–50] cigarettes/day), alcohol consumption (2.5 [0–85] doses/week), HbA1 c (8.4 ± 1.3%), AER (12 [0–15600] mg/24 h), HDL cholesterol (1.64 ± 0.51 mmol/l), LDL cholesterol (2.93 ± 0.81 mmol/l), triglycerides (1.00 [0.20–7.89] mmol/l), and GFR (88.1 [10.3–184.2] ml/min) were entered into a backward multiple regression model with pulse pressure as the dependent variable. The final model is presented below.

| | Coefficient ± SEM | t | P-value |
|---|---|---|---|
| Age, years | 0.68 ± 0.10 | 7.04 | <0.001 |
| Duration of diabetes, years | 0.34 ± 0.05 | 7.61 | <0.001 |
| BMI, kg/m$^2$ | 0.43 ± 0.15 | 2.86 | 0.004 |
| Smoking, cigarettes/day | 0.14 ± 0.06 | 2.27 | 0.02 |
| HbA1 c, % | 0.63 ± 0.38 | 1.66 | 0.10 |
| AER, mg/24 h | 0.002 ± 0.000 | 4.62 | <0.001 |
| GFR, ml/min | –0.08 ± 0.04 | –3.89 | <0.001 |

$R^2$=0.222, adjusted $R^2$=0.216.

**Conclusion:** Age, duration of diabetes, overweight, smoking, and renal damage are independently positively associated with pulse pressure and may therefore contribute to arterial stiffness and the development of cardiovascular disease in type 1 diabetic patients. In contrast, gender, glycemic control, serum lipids, and alcohol consumption does not seem to be associated with pulse pressure.

## 1241

**Estrogen acts on the endothelial function in obese women**

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Obesity is a potent cardiovascular risk factor (CVRF). Compared with obese men, premenopausal obese women exhibit more robust protective endothelial function. The difference is partially contributed to estrogen. This study aims to assess the relationship between the endogenous estrogen and endothelial function in simple obese women and to explore the possible pathophysiological mechanisms.

**Materials and methods:** Ten healthy obese premenopausal women with a mean age of 40.27 ± 6.67 years and 10 BMI-matched healthy obese postmenopausal women with a mean age of 53.7 ± 2.41years were examined in this study. Subjects underwent measurement of insulin sensitivity by the euglycemic hyperinsulinemic clamp technique and measurement of dilatation of brachial artery in response to flow (EDV) and to glyceryl trinitrate (EIV) by ultrasound. All subjects also underwent the measurements of FFAs, MDA(malonic aldehyde), PAI-1(plasminogen activator inhibitor type-1) and NO(nitric oxide).

**Results:** Compared with the obese pretmenopausal women, the obese postmenopausal women had the same insulin sensitivity (9.5 ± 1.69 vs 9.82 ± 1.59 mg/kg/min, P>0.05), the fasting FFAs concentrations (839.3 ± 498.43 vs 721.91 ± 307.46 umol/L, P>0.05), and the change of insulin-mediated nitric oxide release (6.62 ± 10.42% vs 5.38 ± 11.80%, P>0.05). However, the obese postmenopausal women were detected lower endothelium-dependent vasodilation (8.0 ± 3.34% vs 12.52 ± 2.52%, P=0.001), higher PAI-1 activity (0.87 ± 0.1 vs 0.72 ± 0.04, P=0.046), and higher MDA concentrations (2.41 ± 1.88 vs 3.69 ± 2.96 nmol/mL, P=0.502). After adjustment for age and GDR, partial regression analysis revealed a significant positive correlation between EDV and estrogen (r=0.467), and inverse correlation between EDV and FFAs concentrations, PAI-1 activity, and MDA concentrations (r=–0.529, r=–0.346, r=–0.2662, respectively).

**Conclusion:** These results suggest that estrogen in obese premenopausal women can protect the endothelial function. The possible mechanism by which estrogen protects endothelial function may relate to increasing the nitric oxide release and bioactivity by estrogen.

## 1242

**Adiponectin stimulates the production of manganese superoxide dismutase (SOD2) in human aortic smooth muscle cells**

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**Background and aims:** Patients with type 2 diabetes have reduced levels of adiponectin, an adipocyte-derived protein which exhibits anti-atherogenic actions. Previous studies have shown that vascular smooth muscle cells (SMCs) are among the major targets of adiponectin action, and that the anti-atherogenic effects of adiponectin are mediated in part via the inhibition of SMC migration and proliferation induced by growth factors. In this study, we investigated the effects of adiponectin on gene expression in human aortic SMCs.

**Materials and methods:** Human aortic SMCs were treated with bacterially produced full-length adiponectin. cDNA array assay was performed. SOD2 mRNA and protein expression were examined by Northern blot and Western blot analysis, respectively. Cell viability was measured by MTT assay.

**Results:** Data from a cDNA array consisting of only 96 atherosclerosis related genes showed that adiponectin markedly increased the expression of manganese superoxide dismutase (SOD2) in human aortic SMCs. Further studies demonstrated that physiological concentrations of adiponectin induced, dose- and time-dependently, the mRNA expression of SOD2 in human aortic SMCs. Adiponectin also induced SOD2 protein expression and inhibited oxidant $H_2O_2$-induced cell death in human aortic SMCs.

**Conclusion:** These results suggest that adiponectin may act as an antioxidant, at least in part via the stimulation of SOD2 expression and activity. Beneficial effects of adiponectin on oxidative stress may contribute to the reported anti-atherogenic action of rosiglitazone, an anti-diabetic drug which has been shown to increase circulating adiponectin levels in diabetic patients.

## 1243

**Cigarette smoking is associated with reduced adiponectin serum levels in healthy subjects**

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**Background and aims:** Low serum levels of the adipocytokine adiponectin are considered a risk factor for the development of insulin resistance, type 2 diabetes and related disorders like atherosclerosis and coronary artery disease. Preliminary evidence suggests that nicotinic acetylcholine receptors are involved in the regulation of adipocytokine release in adipocytes in vitro. It is unclear whether exposure to exogenous nicotine/smoking is involved in adipocytokine regulation in healthy humans. Therefore, the aim of the present study was to test the hypothesis that cigarette smoking is associated with reduced adiponectin serum levels.

**Materials and methods:** Adiponectin serum levels and data on cigarette consumption were available in 914 (N= 302 males and N= 612 females) healthy non-diabetic subjects (age 36 ± 12 years, BMI 26.1 ± 5.9 kg/m$^2$, percentage body fat 28.4 ± 9.8 % (mean ± SD)). In all subjects insulin sensitivity was estimated from a standard oGTT using a validated index.

**Results:** Adiponectin serum levels were lower in smokers (10.2 ± 0.4 µg/ml (N=193)) compared to non-smokers (12.03 ± 0.3 µg/ml (N=721), p<0.01 after adjusting for sex, age and percent body fat). Insulin sensitivity was comparable between smokers (20.1 ± 0.9) and non-smokers (18.7 ± 0.4, p=0.14 after adjusting for sex, age and percentage body fat).

**Conclusion:** Smoking reduces adiponectin serum levels in a dose dependent manner. The effect on adiponectin serum levels observed with smoking might contribute to the association between cigarette smoking and atherosclerosis. Only longitudinal studies can answer the question whether the individual adiponectin serum level modulates the risk of atherosclerosis and coronary artery disease in smokers.

# PS 130

# Macrovascular disease: genes and molecular mechanisms in humans

## 1244

**Angiotensinogen gene polymorphism and cardiovascular disease in diabetic and non diabetic subjects**

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**Background and aims:** Genetic polymorphisms of Renin-Angiotensin system (RAS) have been implicated in pathogenesis and evolution of cardiovascular disease (CVD) in diabetic and non-diabetic patients. The aim of this study was to evaluate the possible association between angiotensinogen polymorphism regulation and cardiovascular disease.
**Materials and methods:** The study was performed in a random cohort of men from Hospitalet study (Barcelona South District). The cohort were distributed in two groups: 1- Non-diabetic group: control group (n=135), subjects with CVD (n=125), 2- Type 2 Diabetic (T2DM): subjects without (n=125) and with CVD: (n= 100); Classical cardiovascular risk factors, anthropometric measurements and metabolic syndrome (MS) was also defined. ACE insertion/deletion (I/D), angiotensinogen (AGT) (C537T) and angiotensin II type 1 receptor (AT1R) (A1166C) polymorphism genes were evaluated by PCR or RFLP.
**Results:** About 57 % of population with CVD manifested MS ($p<0.0020$, OR 2.68). MS was present in 81.5 % of non-diabetic ($p<0.0012$ vs. controls. OR 2.98) and in 58.4 % of diabetic subjects with CVD ($p<0.043$ vs. diabetics without CVD. OR 1.07). CVD was correlated to classical risk factors presence in the two cohorts of diabetic and in non-diabetic subjects (age, dyslipidemia, hypertension, nicotine, time of diabetes evolution, metabolic control). No association was observed between CVD and I/D (ACE gen) and A1166C (AT1R gen) polymorphisms in the diabetic group, only a weak association was observed with TT genotype presence (C537T. AGT gen). An increase of TT and decrease of MM genotype presence of the AGT gen polymorphism (M237T) were observed in non-diabetic population with CVD (ODD Ratio: 1.89). An interaction between CVD and the presence of DD (ACE I/D polymorphism) and TT genotypes (M237T AGT gen polymorphism) was observed in this population of non-diabetic patients with CVD (ODD Ratio of 2.21).
**Conclusion:** CVD risk factors were observed in non-diabetic and in diabetic subjects with specially mention of MS in non-diabetic population. The TT genotype may be a linkage marker for an etiologic mutation of the AGT gene that confer risk of CVD detectable in non diabetic subjects, however this risk could be quantitatively small among the diabetic male population. Genetic regulation disappears as risk in diabetic subjects and their metabolic control plays a main risk of CVD.
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## 1245

**Intercellular adhesion molecule-1 (ICAM-1) 469 K/E and 241 G/R gene polymorphisms and macroangiopathy in Type 2 diabetic patients**

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**Background and aims:** Adhesion of circulatory leucocytes and monocytes to activated arterial endothelium and subsequent transendothelial migration is one of the earliest events in the pathogenesis of atherosclerosis. In part, this process is mediated by cellular adhesion molecules expressed on the endothelial membrane in response to cytokines. Increased levels of ICAM-1 have been demonstrated in patients with ischemic heart disease, diabetes mellitus and other conditions with an inflammatory component. Two single-base ICAM-1 polymorphisms have been described, changing codon 241 and 469 in the ICAM-1 gene, respectively. To evaluate the role of the ICAM-1 469 K/E and 241 G/R gene polymorphisms as a risk factor for macroangiopathy in patients with type 2 diabetes we performed a study in more than 400 patients.
**Materials and methods:** A total number of 427 patients was included (214 with type 2 diabetes). There were no significant differences with regard to basic variables and allele distribution. All patients were genotyped by PCR and allele specific oligonucleotide technique for ICAM-1 polymorphism G/R at codon 241 and K/E at codon 469 was performed.
**Results:** In the subgroup of patients with type 2 diabetes there was no association between the ICAM-1 469 and 241 genotype and the presence or extent of coronary artery disease (CAD). In contrast, patients without diabetes and at least one R allele of the 241 polymorphism or homozygosity for the K allele of the 469 polymorphism had an increased prevalence of CAD. The analysis of interaction between both gene polymorphisms showed a strong linkage disequilibrium.
**Conclusion:** The obtained data show that both polymorphisms are not of importance for macroangiopathy in patients with type 2 diabetes. However, in nondiabetic patients the R allele of the 241 and the K allele of the 469 polymorphism seem to favor the occurance of CAD and might therefore be regarded as risk factor. This striking difference between diabetic and non-diabetic patients might be due to other non conventional, more severe risk factors that overweight in the group of diabetic patients.

## 1246

**Monocyte-chemoattractant-protein-1 (MCP-1) represents a risk factor for elevated intima-media-thickness (IMT) in insulin resistance**

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**Background and aims:** The increased morbidity and mortality of type 2-diabetes mainly results from the accelerated atherosclerotic processes in this group of patients. It is undoubted that inflammation plays a central role in the development of atherosclerosis. In this context, the MCP-1 mediated adhesion of monocytes represents an important mechanism. Recently, serum MCP-1 concentrations were reported to be associated with insulin resistant states.
We tested the hypothesis, whether elevated MCP-1 plasma levels are associated with early stages of atherosclerosis, such as elevated IMT, in relation to insulin sensitivity in a young according to current definition healthy study population.
**Materials and methods:** We performed an oral glucose tolerance test in 148 patients with 75 g glucose and simultaneous insulin measurements in order to assess the insulin-sensitivity-index (ISI) according to the criteria described by Matsuda. The IMT was measured by the means of high resolution ultrasound (13 MHz) at the common carotid artery. MCP-1 levels were measured by ELISA.
**Results:** The mean age of the study population was 37,3 ± 11,1 years with a mean body-mass-index (BMI) of 27,9 ± 6,0 kg/m$^2$. Blood pressure was 128 ± 16 to 79 ± 12 mmHg. The measured MCP-1 levels and IMT were 164,9 ± 84,83 pg/ml and 0,54 ± 0,12 mm, respectively.
We found a significant positive correlation between MCP-1 and IMT ($r=0,346$; $p<0,0001$), as well as between MCP-1 and ISI ($r=0,310$; $p<0,001$). Furthermore, there was a distinct relation between MCP-1 and other metabolic syndrome typical parameters like HDL, trigylcerides, BMI, age, and blood pressure, except LDL. In the multivariate analysis low HDL and higher age turned out to be independent predictors for MCP-1 and IMT.
**Conclusion:** We conclude that MCP-1 might represent a relevant link between insulin resistance and developing atherosclerosis. In this respect, the typical dyslipoproteinemia pattern of the metabolic syndrome seems to have a triggering function.

## 1247

**Hypertriglyceridemic hyperapo B is not related to atherosclerosis in the Korean Type 2 diabetes**

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**Background and aims:** Atherosclerotic disease such as cardiovascular and cerebrovascular disease are major cause of diabetes mellitus-related morbidity and mortality. The frequency of macrovascular disease in type 2 diabetic patients varies geographically, suggesting that factors other than diabetes play an important role in the pathogenesis of the vascular disease. One such factor may be the dyslipoproteinemias that are common in diabetic patients. There were many studies that hypertriglyceridemia with an elevated apo B level was associated with increase in risk for coronary disease in type 2 diabetes. Meanwhile, an increase in the intima-media thickness (IMT) of the carotid artery has previously been reported in patients with type 2 diabetes, and is related to atherosclerotic risk factors. The aim of the study was to evaluate the relationship between the carotid artery IMT and lipoprotein and apolipoprotein and to assess the role of

hypertriglyceridemic hyperapo B for the cardiovascular risk factors in the Korean type 2 diabetic patients.
**Materials and methods:** The carotid artery IMT was measured using high resolution B-mode ultrasonography in 117 Korean type 2 diabetes. At the same time, we analyzed patient's characteristics including height, weight, body mass index, blood pressure, duration of diabetes, and history of hypertension. Laboratory parameters such as fasting blood glucose, HbA1 c, total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol, apolipoprotein A, apolipoprotein B, were included in this study. We defined hypertriglyceridemic hyperapo B when the triglyceride level was over than 150 mg/dl and apolipoprotein B level was over than 115 mg/dl.
**Results:** Thirty-three (28%) were classified hypertriglyceridemic hyperapo B. In a simple correlation analysis, age ($r=0.398$, $p<0.001$), duration of diabetes ($r=0.513$, $p<0.001$), blood pressure ($r=0.354$, $p<0.002$) were statistically significant for the carotid artery IMT value in total diabetic patients. But, there were no correlation between carotid artery IMT and lipoprotein and apolipoprotein. In hypertriglyceridemic hyperapo B diabetic patients didn't have higher carotid artery IMT value than others.
**Conclusion:** Increment of carotid artery IMT is affected by age, blood pressure and duration of diabetes such as atherosclerotic risk factors. However, our study did not show association between carotid artery IMT and hypertriglyceridemic hyperapo B. In Korean type 2 diabetes, hypertriglyceridemic hyperapo B might does not major role in development of atherosclerosis.

## 1248

**Elevated anti-ORP150 autoantibody associates with early stage carotid atherosclerosis in Type 1 diabetes**
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**Background and aims:** It is well known that various genetic and environmental stresses interfere with protein folding in endoplasmic reticulum (ER), leading to induction of ER stress. 150 kda oxygen-regulated protein (ORP150), a molecular chaperone located in the ER, is induced by ER stress, and reduces ER stress. Local expression of ORP150 is observed in initial stages of atheroma in high fat diet fed mice and atherosclerotic plaques in humans. Circulating anti-ORP150 autoantibody was elevated in these mice and humans. Therefore, enhanced ER stress may contribute to the establishment and progression of atherosclerosis. In this study, we investigated the relationship between the serum value of anti-ORP150 autoantibody and the carotid IMT in young type 1 diabetes patients, then determined the contribution of the ER stress to the development of atherosclerosis.
**Materials and methods:** The mean IMT of the carotid artery was assessed using ultrasound B-mode imaging in 77 patients with type 1 diabetes (29 men and 48 women, aged 24 ± 4.8 years, duration of diabetes 14 ± 5.3 years) and 24 age-matched healthy non-diabetic subjects (10 men and 14 women, aged 24 ± 3.2 years). Anti-ORP150 autoantibody was measured with ELISA and high-sensitive C-reactive peptide (hs-CRP) was determined by a latex-enhanced immunonephelometer.
**Results:** Anti-ORP150 autoantibody and high-sensitive C-reactive peptide (hs-CRP) in type 1 diabetes was three fold higher than those in non-diabetic subjects. The mean IMT was significantly correlated with hs-CRP and anti-ORP autoantibody in patients with type 1diabetes (mean IMT vs. hs-CRP $P<0.05$, mean IMT vs. anti-ORP autoantibody $P<0.05$) but not non-diabetic subjects. Anti-ORP150 autoantibody was not significantly correlated with age, gender, blood pressure, HbA1 c, duration of diabetes, and diabetic complications. Also, anti-ORP150 autoantibody was not correlated with inflammatory markers, such as hs-CRP, and immunoglobulin G for *Cytomegalovirus*, *Helicobacter pylori* and *Chlamydia*.
**Conclusion:** We firstly demonstrated that circulating anti-ORP150 autoantibody levels were elevated in young patients with type 1 diabetes, and were correlated with early stage of atherosclerosis. These data suggest that ER stress may contribute to early-stage carotid artery atherosclerosis independently of inflammation and reported common risk factors of atherosclerosis.

## 1249

**Antioxidant enzyme activity, vitamin E, and fatty acid levels in African-Caribbean and Caucasian patients with Type 2 diabetes mellitus**
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**Background and aims:** An increase in oxidative stress has been associated with diabetes mellitus complications. This may be a factor in the increased prevalence of nephropathy in patients of African-Caribbean (AC) origin. We have previously reported an increase in lipid hydroperoxide concentration in (AC) compared with Caucasian type 2 diabetes patients. We investigated whether there were racial differences in antioxidant enzyme activity, vitamin E, and some polyunsaturated fatty acids which play a protective role in diabetic nephropathy
**Materials and methods:** Eighty type 2 diabetes patients of AC (n=34) and CA (n=46) origin were studied. The mean [SD] age and duration of diabetes tended to be higher in the AC compared with the CA group (66.7[8.5] vs 62.9[8.4] yrs; $p=0.05$ and 16.4[9.5] vs 12.2[7.7] yrs; $p=0.04$). Body mass index, systolic blood pressure, albumin excretion ratio and glycaemic control were similar in the AC and CA groups 28.4[2.9] vs 30[4.8]; $p=0.09$, 150.9[23.9] vs 142.8[19.8]; $p=0.10$, 121.3[339.4] vs 36.3[62.4] mg/mmol; $p=0.83$ and 8.0[1.3] vs 77[1.6]%, $p=0.33$. Fasting venous blood was collected for the measurement of plasma α-tocopherol by HPLC and antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase activities spectrophotometrically. Fatty acids, arachidonic acid (AA), linoleic acid, and eicosapentaenoic (EPA) acid were analysed by Gas Chromatography
**Results:** Table. Antioxidants and fatty acid levels in type 2 diabetes patients from African-Caribbean and Caucasian origin.

| Parameters | African-Caribbean | Caucasian | P |
|---|---|---|---|
| SOD (u/l) | 721.86 [589.99] | 445.33 [419.66] | 0.002 |
| GPx (u/l) | 125.72 [82.26] | 171.9 [97.49] | 0.02 |
| Catalase (Ku/l) | 30.90 [41.74] | 31.28 [37.01] | 0.09 |
| α-tocopherol (umol/l) | 31.69 [9.67] | 37.81 [13.67] | 0.05 |
| α-tocopherol/Total cholesterol (umol/mmol) | 6.2 [1.64] | 7.81 [2.78] | 0.01 |
| AA (%) | 80.29 [4.71] | 82.41 [3.39] | 0.05 |
| Linoleic acid (%) | 15.38 [5.42] | 15.55 [3.09] | 0.83 |
| EPA (%) | 4.13 [3.47] | 2.02 [0.83] | 0.01 |

Data presented as: mean[SD]

**Conclusion:** These data suggest that racial differences exist in the activity of antioxidant enzymes and α-tocopherol concentrations in patients with type 2 diabetes and may contribute to increased oxidative stress status in African-Caribbean type 2 diabetes patients

## 1250

**Arterial osteoprotegerin: increased amounts in diabetes and modifiable synthesis from vascular cells by insulin and TNF-alpha**
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Extracellular matrix modifications and linear medial calcifications are elements of the diabetic macroangiopathy. The bone-related protein osteoprotegerin (OPG) has been found in the arterial matrix and may be associated with the development of vascular calcifications. In the present study, we first measured the amount of OPG in the thoracic aorta, obtained at autopsy from 21 diabetic and 42 gender- and age-matched controls. Intima and media samples from normal and fibrous plaque areas from the individual vessels were evaluated. OPG was estimated in tissue extracts by an ELISA and was found in statistically significantly higher amounts in media samples from diabetic individuals, when compared to specimens from non-diabetics ($p<0.05$). This was observed both in normal and plaque areas, however no differences between diabetic and non-diabetic subjects were observed, when intimal tissue compartments were compared. In the second part of this investigation, the production of OPG was estimated from vascular cells in vitro. Human vascular smooth muscle cells (HVSMC) produced much higher amounts than human umbilical vein endothelial cells. Insulin addition to HVSMC was followed by a dose- (50–1000 μU/ml) and time- (12–48 hours) dependent decrease in OPG production (maximal

effect: approx. 60 % of control, $p < 0.01$), whereas TNF-α supplement gave rise to increased amounts in a time- (12 – 48 hours) and dose- (0.04 – 1 ng / ml) dependent manner (maximal effect: approx. 200 % of control, $p < 0.01$)). Alterations in OPG production by insulin and TNF-α was paralleled by similar effects on the mRNA expression for OPG. Addition of growth hormone (10 ng/ml) or extra glucose (25 mM) to the growth medium did not alter OPG secretion. Increased OPG concentrations in the arterial wall in diabetes may be a part of generalized matrix alterations, putatively related to the development of arterial calcifications. Altered arterial OPG content in diabetes may be a consequence of the effects of hormones and cytokines, like insulin and TNF-α.

## 1251

**Insulin generates free radicals a NAD(P)H , phosphatidylinositol-3'-kinase dependent mechanism in human skin fibroblasts ex vivo**

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**Background and aims:** Oxidative stress may be involved in the development of vascular complications associated with diabetes: however, the molecular mechanism for increased free radicals production in diabetes remains uncertain. Therefore, we examined whether the acute hyperinsulinemia may increase free radicals production and whether this condition affects proliferative extracellular signal-regulated protein kinases (ERK1/2) signalling in human fibroblasts in vitro.
**Materials and methods:** The free radical production in fibroblasts from 6 human volunteers was investigated using Electron Spin Resonance (ESR) spectroscopy with the spin clearance method and nitroxide free radical used (acetoxymethyl- 2,2,6,6-tetramethylpiperidine-1-oxyl-3-carboxylate, CT-AM) as a probe. The probe CT-AM (20 µmol/L) and insulin (from 1 nmol/L to 10 µmol/L) were added to the cell suspensions. In some experiments, cell suspensions were incubated for 1 hour at 37°C with one of the following inhibitors before the stimulation with insulin (1 µmol/L): apocynin (100µmol/L), LY294002 (100µmol/L), rotenone (1 mmol/L) or oxypurinol (1 mmol/L). In the experiments aimed at the identification of the molecular species responsible for CT-AM elimination, the fibroblasts were pretreated with the superoxide radical scavenger Tiron (1 mmol/L), or with PEG-SOD (250U/mL), or with PEG-catalase (250U/mL), before insulin stimulation. The protein expression of p47phox and the immunoblot analysis of Extracellular regulated kinases 1 and 2 phosphorylation were determined
**Results:** Treatment with insulin significantly increased intracellular superoxide anion ($O_2^-$) production: this effect was completely abolished by Tiron, a cell-permeable SOD mimetic and by PEG-SOD, but not by PEG-catalase. Furthermore, insulin-induced $O_2^-$ production was attenuated by NAD(P)H inhibitor, apocynin, but not by rotenone and by oxypurinol. Inhibition of phosphatidylinositol 3'-kinase (PI3K) pathway with LY294002 blocked insulin-stimulated $O_2^-$ production, suggesting a direct involvement of PI3K in the activation of NAD(P)H oxidase. The insulin-induced free radical production led to membranous translocation of p47phox and markedly enhanced ERK1/2 activation in human fibroblasts.
**Conclusion:** These findings provide direct evidence that elevated insulin levels generate $O_2^-$ by a NAD(P)H dependent mechanism, which involves the activation of PI3K and stimulates ERK1/2 dependent pathways. This effect of insulin may contribute to the pathogenesis and progression of cardiovascular disease in the insulin resistance syndrome.

## 1252

**Glucose alters platelet-derived growth factor-BB activity in human aortic vascular smooth muscle cells by stimulating protein phosphatase 2A in a protein kinase C-beta II-dependent pathway**

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**Background and aims:** Diabetes is associated with a premature onset of atherosclerosis. Platelet-derived growth factor (PDGF) is elevated in atheromas and is implicated in the phenotypic switching and migration of vascular smooth muscle cells (VSMC) into plaques. The aim of this work is to assess the interactions of glucose and PDGF-BB on PDGF-beta receptor upregulation and directed migration of explant-derived human aortic VSMC.
**Materials and methods:** The level of the PDGF-beta receptor and second messenger proteins was assessed using immunoprecipitation and Western blot, or kinase assays for downstream target molecules. Directed cellular migration (chemotaxis) was recorded using the Dunn chamber and analysed by cyclical statistics.
**Results:** At physiologically relevant levels of PDGF-BB (<15 ng / ml), 25 mmol / l (HG) glucose for 24 h causes an increase in the concentration of PDGF-beta receptors in cultured VSMC ($p<0.0001$, 2-way ANOVA vs. 5 mmol / l [NG] glucose). At higher levels of PDGF-BB (>15 ng / ml) in HG there is a reduction in receptor concentration. There is therefore a biphasic response in receptor concentration as PDGF-BB level increases in HG.
It is known that phosphatases may be activated by high levels of growth factors. At high levels of PDGF-BB endothall (90 nmol/l), the protein phosphatase 2A (PP2A) inhibitor but not PTP1B, PP1, or PP2B inhibitors, caused an increase in PDGF-beta receptor concentration and a loss of biphasicity in receptor concentration in HG. The receptor response in HG was accompanied by activation of the MAPK pathway (MAPK activity assay) and phosphorylation of the MAPK pathway target ERK. Addition of the ras farnesylation inhibitor Lovastatin (10 nmol/l), or the MEK1/2 inhibitor PD98059 (10 micromol/l), reduced phosphorylation of ERK and PDGF-beta receptor protein concentration at both low (10 ng/ml) and elevated (50 ng/ml) levels of PDGF-BB ($p<0.01$ vs. HG alone in all cases). ERK activation increased in HG conditions in the presence of the PP2A inhibitor in 50 ng/ml PDGF-BB.
HG is known to activate PKC, but only PKC-beta II is significantly raised at 24 h in HG conditions in human aortic VSMC ($p<0.01$ vs. NG). In the presence of the PKC-beta II inhibitor (LY379196; 200 nmol/l) the PDGF-beta receptor protein concentration at 10 ng / ml PDGF-BB was decreased ($p<0.01$ vs. HG) but at 50 ng/ml it was significantly increased ($p<0.01$ vs. HG). A similar response was also found for ERK phosphorylation ($p<0.01$ vs. 50 ng/ml PDGF-BB in HG).
Since PP2A and PKC-beta II inhibition both recovered the receptor level and ERK activation level in HG ($p<0.01$ vs. HG alone in both cases), the effect of the PKC-beta II inhibitor on activation of PP2A was examined by immunoprecipitation. PP2A activation, elevated in HG conditions at 50 ng/ml PDGF-BB ($p<0.05$ vs. NG), was significantly decreased in the presence of LY379196 ($p<0.05$ vs. HG alone).
Chemotaxis occurred in HG conditions in a 10 ng/ml PDGF-BB gradient but could be blocked by the addition of Lovastatin, PD98059 or LY379196. At the elevated concentration of PDGF-BB (50 ng/ml) in HG, the chemoattractant effect of PDGF-BB was lost, however inhibition of PP2A restored chemotaxis.
**Conclusion:** The biphasic response in PDGF-beta receptor level and in chemotaxis to PDGF-BB in HG is due to PP2A activation in response to upregulation of PKC-beta II activity.

# PS 131

# Macrovascular disease: effects of pharmacotherapy

## 1253

**Direct effects of simvastatin on vascular smooth muscle responsiveness of diabetic rats: role of calcium mobilization**

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**Background and Aims:** Simvastatin, a 3-hydroxy-3-methyglutaryl coenzyme A (HMG-CoA) reductase inhibitor, is widely used in the treatment of both diabetic and non-diabetic dyslipidemia. Recent studies suggested that the actions of HMG-CoA reductase inhibitors in the vasculature are attributable to more than their cholesterol lowering effect. To clarify the role of calcium homeostasis in the mechanisms of direct effects of simvastatin on vascular responsiveness, we examined the effects of simvastatin on phenylephrine (Phe) and calcium ($CaCl_2$)-induced contractions in aortae from diabetic and non-diabetic rats.
**Materials and Methods:** Two groups of Wistar rats were used as diabetic and their age-matched controls. Diabetes was induced by a single intraperitoneal injection of streptozotocin (45 mg/kg). Blood pressure levels (by tail-cuff method), blood glucose, triacylglycerol and total cholesterol concentrations were measured. After 8 weeks, thoracic aortae were removed and mounted in organ baths for isometric tension determinations. In calcium-free medium, Phe- ($10^{-6}$M) and $CaCl_2$ ($10^{-2}$M) -induced contractions were evaluated in the presence or absence of simvastatin ($10^{-6}$M), simvastatin + mevalonate ($10^{-6}$M+$10^{-6}$M), thapsigargine (sarcoplasmic $Ca^{2+}$-ATPase inhibitor;$10^{-6}$M) or nifedipine ($Ca^{2+}$ channel blocker; $10^{-6}$M ) in endothelium intact aortic rings. Inhibitors were added to the bath 10 min before contractile responses were obtained.
**Results:**
Incubation of simvastatin reduced Phe- and $Ca^{+2}$ -induced contractions which increased significantly in diabetic aorta and this effect was reduced by mevalonate. All the contractile values and other measured parameters are shown in Table 1 and Table 2 respectively.

Table 1: Maximum Contractile Responses After Incubations (mg tension)

| | Simvastatin | Simvastatin + Mevalonate | Thapsigargine | Nifedipine | No incubation (NI) |
|---|---|---|---|---|---|
| **Diabetic** (Phe Contraction, n=12) | 378.33 ± 38.10## | 543.20 ± 20.12 | 434.00 ± 20.00 | 526.50 ± 12.11 | 571.67 ± 40.01 |
| **Control** (Phe Contraction, n=8) | 94.86 ± 24.31## | 457.00 ± 15.00# | 349.33 ± 14.22 | 308.50 ± 12.10## | 397.40 ± 12.12 |
| **Diabetic** ($CaCl_2$ Contraction, n=12) | 309.57 ± 27.82†† | 385.00 ± 11.55 | 312.50 ± 12.50†† | 343.10 ± 5.81† | 400.00 ± 12.99 |
| **Control** ($CaCl_2$ Contraction, n=8) | 126.25 ± 24.61†† | 270.50 ± 8.00 | 257.50 ± 7.50 | 250.33 ± 3.93 | 285.11 ± 12.99 |

Table 2: Some Parameters of Experimental Groups

| | Blood Pressure (mm Hg) | Blood Glucose (mg/dl) | Blood Triacylglycerole (mg/dl) | Blood Total Cholesterol (mg/dl) |
|---|---|---|---|---|
| **Diabetic** (n=12) | 138 ± 11* | 456 ± 10* | 118 ± 9* | 120 ± 8* |
| **Control** (n=8) | 109 ± 10 | 100 ± 5 | 48 ± 8 | 45 ± 6 |

##p<0.01 and #p<0.5*vs.* diabetic NI.
††p<0.01 and †p<0.5*vs.* control NI.
* p<0.001 *vs.* control.

**Conclusion:** Our results suggest that simvastatin affects calcium homeostasis in vascular smooth muscle. This effect partly may be related to the inhibition of isoprenoid synthesis through mevolanate-dependent pathway in both diabetic and non-diabetic rat vascular smooth muscle.

## 1254

**Vasculoprotection by metformin is exerted by its anti-oxidative properties**

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**Background and aims:** There is evidence that metformin (M) exerts a vasculoprotective effect independent of its well known antihyperglycemic action. It has been suggested that an antioxidative action is of major importance for vasculoprotection exerted by M.
**Materials and methods:** The antioxidative effect of M was studied in human endothelial cells (HUVEC) after pre-incubation of the cells with M (up to 1 mM for 48 hrs). To induce ROS formation cells were incubated with glucose (up to 30 mM), palmitic acid (100 and 250 µM, or AGEs for 1 hr. The formation of reactive oxygen species (ROS) was measured by confocal microscopy after loading the cells with dichlorodihydrofluorescein method (DCF, 1 hr). For the in vivo study streptozotocin (60 mg/kg body weight) diabetic (as model for type 1 diabetes) and Goto-Kakizaki (as model for type 2 diabetes) rats were pre-treated with M for 8 weeks (200 mg/kg body weight). Thereafter the lipidhydroperoxides, the carbonyls and the total antioxidant activity in plasma were determined by standard techniques. NADH-oxidase activity in aortae was measured by the Lucigenin method. The endothelium dependent relaxation was determined by measuring the acetylcholine dependent relaxation of isolated in aortae.
**Results:** Incubation of HUVEC with glucose, AGEs, or palmitic acid caused a dose dependent increased in ROS indicated by DCF fluorescence. This increase was significantly reduced by pre-incubation of the cells with M. Induction of diabetes in rats increased the amount of lipidhydroperoxides and carbonyls in plasma (2.3 ± 1.3 vs 5.3 ± 0.8 and 4.6 ± 0.2 vs 20.9 ± 5.3, $p < 0.05$), an effect which was not prevented by pre-treatment of the rats with M. However, the total antioxidant activity reduced in diabetes (2.3 ± 1.3 vs 1.3 ± 0.6, $p < 0.05$) was increased by M 4 fold ($p<0.05$). In Goto-Kakizaki rats M reduced the rise in blood glucose and the manifestation of diabetes. However M had no significant influence on the amount of lipidhydroperoxides and total antioxidant activity in plasma and did not change the activity of NADPH oxidase. However, endothelium (NO) dependent vasodilation was significantly improved by pre-treatment with M.
**Conclusion:** These data indicate that M exerts an direct antioxidative action on the endothelium and increases the bioavailability of nitric oxide. This antioxidative effect is not caused by an inhibition of NADPH-oxidase, but compatible with a tightning of the electron flux in mitochondria. The observation that in plasma the formation of ROS was increased in diabetes, but not affected by M, although the total antioxidant activity was improved, suggests that M is not effective against the generation of ROS by oxidative processes in blood, but exerts its antioxidative effect specifically in the vasculature.

## 1255

**Effect of gliclazide on atherogenic biological events associated with the process of human monocyte differentiation into macrophages**

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**Background and aims:** As monocytes differentiate into macrophages, they show increased scavenger receptor expression and endocytotic activity of modified lipoproteins as well as enhanced sensitivity towards lipopolysaccharide (LPS). Acquisition of these functional properties plays a critical role in foam cell formation and macrophage activation, two key features of atherogenesis. We previously demonstrated that gliclazide, a sulphonylurea with antioxidant properties, decreases agonist-induced endothelial and smooth muscle cell activation. In the present study, we investigated the in vitro effect of gliclazide on human monocyte-derived macrophage foam cell formation and LPS-induced cytokine production.
**Materials and methods:** Freshly isolated human monocytes were differentiated into macrophages in the presence or absence of gliclazide (1–10 µg /ml) or vitamin E (50 µM). After 9 days of culture, the expression and activity of the macrophage scavenger receptor CD36 was determined by PCR analysis and uptake of Dil-labelled minimally modified LDL (Dil-mmLDL) (80 µg /ml), respectively. mmLDL-induced foam cell formation was assessed by staining the cells with oil red O and measuring the optical

density of the extracted dye. LPS-stimulated tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6) secretion by macrophages was determined by ELISA.
**Results:** Differentiation of human monocytes into macrophages in the presence of gliclazide (1–10 µg/ml) decreased CD36 expression by 20 to 50%, with maximal effect occurring at 2.5 µg/ml ($P < 0.05$). This effect was mimicked by vitamin E. Incubation of the cells with gliclazide also reduced CD36 activity by 30% ($P < 0.02$). Despite these effects, neither gliclazide nor vitamin E did affect foam cell formation. In contrast, gliclazide significantly reduced LPS-stimulated macrophage TNFα and IL-6 secretion ($P < 0.05$).
**Conclusion:** These data demonstrate that gliclazide, at concentrations in the therapeutic range, reduces monocyte-derived macrophage CD36 expression and activity, although it did not affect foam cell formation. This drug also reduces LPS-induced macrophage activation. Overall, these data indicate that gliclazide may regulate some key biological events associated with the process of monocyte differentiation into macrophages. By doing so, it may help reduce diabetic macrovascular complications.

## 1256

**Cilostazol inhibits high glucose-stimulated increase in E2F expression and proliferation in human aortic vascular smooth muscle cells *in vitro* and *in vivo***

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**Background and Aims:** A selective type 3 phosphodiesterase (PDE3) inhibitor, Cilostazol, has potent vasodilator properties and anti-proliferative action on the growth of vascular smooth muscle cells (VSMC). Although it is established that Cilostazol suppresses the growth of VSMCs *in vitro* and *in vivo*, little is known about its molecular mechanism of action. In the present study, we focus on the effect of cilostazol on an important cell cycle transcription factor, particularly the positive regulator, E2F, in VSMC proliferation.
**Materials and Methods:** We evaluated the effects of cilostazol on high glucose-induced VSMC proliferation *in vitro* and neointimal formation in an *in vivo* model of rat carotid artery injury. We additionally measured promoter activity and DNA binding activity of E2F, as well as protein expression of E2F1, E2F2, E2F4 and PCNA.
**Results:** Cilostazol inhibited VSMC proliferation in response to high glucose *in vitro*, and virtually abolished neointimal formation after balloon injury in rats subjected to carotid artery injury *in vivo*. Moreover, the compound suppressed high glucose-induced E2F promotor activity, E2F binding activity, and expression of E2F1, E2F2, E2F4 and PCNA proteins.
**Conclusion:** Cilostazol inhibits high glucose-induced E2F expression and proliferation of VSMCs. Based on the data, we propose that the anti-proliferative effect of cilostazol on VSMCs is mediated, at least in part, by inhibition of E2F activity.

## 1257

**High dose folic acid significantly lowers blood pressure , central arterial stiffness and plasma homocysteine levels in Type 2 diabetes but not non diabetic subjects. A randomised double blind control study**

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**Background and aims:** Homocysteine is associated with atherothrombotic disease, which may be mediated through associations of homocysteine levels with blood pressure, endothelial function or arterial stiffness
**Materials and methods:** In a placebo-controlled, randomized clinical trial, we measured blood pressure, homocysteine levels and indices of central arterial stiffness in 50 patients T2D (mean age 54yrs ± 2.4, 14F ) diagnosed with type 2 diabetes and 20 healthy individuals ND (53.0 yrs ± 1.8, 10F), at baseline and after 28 days of treatment with folic acid (5 mg).
**Results:** Compared to baseline; treatment with folic acid resulted in a significant reduction in homocysteine levels in T2D (14.6 µmol / L ± 3.0 V 16.6 µmol/L ± 3.0 $p< 0.0005$) and ND (8.6 µmol/L ± 0.4 V 9.9 µmol / L ± 0.50 $p< 0.0005$). Treatment with folic acid was associated with lower brachial systolic blood pressure (137 mmHg ± 2.2 V 145 mmHg ± 3.7 $p<0.03$) and aortic systolic blood pressure (130 mmHg ± 3.4 V 123 mmHg ± 2.0 $p<0.02$) in only T2D but not ND. Decreased augmentation index (AI) a marker of central arterial stiffness was observed in T2D (23.6% ± 2.9 V 19.7% ± 3.4 $p<0.05$) but not ND.
**Conclusion:** Our results support the hypothesis that homocysteine lowering treatment with folic acid has beneficial vascular effects in type 2 diabetics.
*Supported by: Abbott Pharmaceuticals*

## 1258

**Effect of insulin glargine versus regular human insulin on human coronary artery endothelial and smooth muscle cells**

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**Background and aims:** The role of insulin and insulin analogues (e.g. insulin glargine [LANTUS®]) in the pathogenesis of diabetes-associated atherosclerosis is controversial. Therefore, the objective of the present study was to compare proliferative and apoptotic effects of insulin glargine, a long-acting human insulin analogue, with regular human insulin in endothelial cells (CAEC) and smooth muscle cells (CASMC) from human coronary arteries.
**Materials and methods:** CAEC and CASMC (Clonetics/BioWhittaker) were cultured according to the manufacturer's instructions. Cells were treated with regular human insulin or insulin glargine for 20 hrs and proliferation was determined by 3[H]thymidine incorporation and flow cytometric analysis of Ki-67 expression. Apoptosis was assessed after 24 hrs by flow cytometry (cell cycle analysis and Annexin V-staining).
**Results:** Treatment of CAEC and CASMC with regular human insulin or insulin glargine did not significantly promote cell growth. There was no significant increase in DNA synthesis and Ki-67 expression with either insulin. Notably, incubation of CAEC and CASMC with insulin glargine or regular human insulin for 24 hours did not increase the number of apoptotic cells. Furthermore, no influence of insulin glargine or regular human insulin on lipoapoptotic vascular cell death could be detected.
**Conclusion:** Taken together, neither regular human insulin nor insulin glargine promote the proliferation or apoptosis of human coronary artery cells in vitro. There is no evidence from our in vitro data that regular human insulin or insulin glargine may promote atherosclerosis via increased proliferation or apoptosis of human coronary artery cells.

## 1259

**Concept of the Type 2 diabetes polypill: statin, aspirin, metformin, thiazide and ACE-I or ARB (SAMTA-pill)**

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**Background and aims:** There is now considerable evidence to show that *S*tatins, *A*spirin, *M*etformin and antihypertensive agents such as *T*hiazides and *A*CE Inhibitors (or Angiotensin receptor blocker) can reduce morbidity and mortality due to cardiovascular disease. We could theoretically at least suggest that many patients with Type 2 Diabetes would benefit from the SAMTA combination of therapies. We have called this the SAMTA pill because Indo-linguistically SAMTA means equality and SAMTA pill may be a method of equalising the increased cardiovascular morbidity and mortality in diabetes to that of the non diabetic population. The aim of this study was to look at the prescribing of the SAMTA drugs within a cohort of 200 patients with Type 2 diabetes and hypertension. Data was analysed from a prospective audit of blood pressure (BP), cholesterol and glycaemic control management after the implementation of the Alphabet Strategy. The Alphabet Strategy is diabetes care based around the mnemonic: A – advice, B – BP control, C – cholesterol profile, D –diabetes control, E – eye examination, F – foot examination, G – guardian drugs (aspirin, ACE-inhibitors or ARB, statins).
**Materials and methods:** Diabetes and cardiovascular parameters were collected from 200 randomly selected patients with established hypertension from the diabetes database. Most patients did not have previous CHD. Data were collected at diagnosis of hypertension (T0) and from the last clinic visit (T1). Data were analysed using Student's paired t-test and the chi-squared test.
**Results:** The mean duration of follow-up was 3.8 years. Blood pressure improved significantly (SBP: 163.65 ± 18.74 mmHg vs. 146.50 ± 21.19 mm Hg; DBP: 88.44 ± 11.31 mmHg vs. 77.68 ± 10.13 mmHg. $p < 0.0001$). UKPDS cardiac risk score was initially 29.2%, improving to 26.0% at follow-up. The prescribing of the various components of the "SAMTA-pill" were as follows: SAMTA-pill: All 5 components 21.5%, Any 4 components 57%, Any 3 components 91.5% amongst which Statin+Aspirin+ACEI was 48.5% and Statin+Aspirin+Metformin was 38%, Any 2 component 99% amongst

which Statin+Aspirin combination was 59.5% and combination of ACE-I or ARB +Thiazide was 55.5%
By individual component: Statin 67%, Aspirin 89.5%, Metformin 64.5%, Thiazide 72.5% and ACE inhibitor or ARB 79%
**Conclusion:** The SAMTA pill offers the prospect of considerable reduction in cardiovascular morbidity and mortality in a primary prevention cohort of patients with Type 2 Diabetes. Our study confirms that the drugs were well tolerated with potential for increased prescribing of some or all of the components of the SAMTA pill.

## 1260

**Steno-2 study data supports the polypill concept with respect to cardiovascular risk reduction**
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**Background and aims:** The radical Polypill concept stated that cardiovascular risk can be reduced by 88% through intensive polypharmacy addressing cardiovascular risk factors. This concept is based on a theoretical epidemiological model. Our study aimed to ascertain whether 50% reduction in coronary heart disease (CHD) in Steno-2 through multi-factorial pharmacological intervention could be modelled using the Polypill concept.
**Materials and methods:** CHD risk according to the UKPDS Risk Engine for Steno-2 intensive cohort baseline multiplied by 2 (for microalbuminuria effect) = 72.4/100 subjects/10-years.
Predicted relative risk reduction (RRR) in the Steno-2 study using the Polypill model:
§ Steno-2 actual LDL-C reduction was 1.215 mM. Predicted Polypill LDL-C reduction = 1.8 mM, with relative risk (RR) of 0.39. We would therefore expect 1.215/1.8 = 67.5% of the Polypill RR. 67.5% of 61% (Polypill RR) = 41%. Therefore CHD RRR is 0.59.
§ Steno-2 Diastolic BP reduction = 12 mmHg. Polypill predicted reduction = 11 mmHg, with RR of 0.54. We would expect 12/11 = 109.1% of Polypill RR. 109.1% of 46% (Polypill RR) = 50%. Therefore CHD RRR is 0.5.
§ Aspirin usage 72.5% in Steno-2. Polypill usage = 100%, with RR of 0.68. We would expect 72.5/100 = 72.5% of Polypill RR. 72.5% of 32% (Polypill RR) = 23.3%. Therefore CHD RRR is 0.77.
§ Combined product of above 3 RRR (0.59x0.5x0.77)=0.227.The combined CHD RRR is therefore 77.3% ((1–0.227)x100).
**Results:** The actual observed Steno-2 event rate was 18.8/100 patients/10-years, applying the Polypill Concept would give an event rate 16.4 (expected 72.4 from risk engine reduced by 77.3% = 16.4). Similarly, the conventionally treated cohort had an event rate of 37.5/100 patients/10-years, compared to a Polypill predicted event rate of 40.8.
**Conclusion:** The close correlation of actual Steno-2 and Polypill-predicted event rates may offer a "Proof of Concept" for the Polypill. The Polypill or "Aspostatinoprilololazide folate" may offer a realistic opportunity to reduce cardiovascular complications in patients with type 2 diabetes.

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## 1261

**Inhibitory effects of rosiglitazone on activation of NF-κB and expression of MCP-1 in rat mesangial cells**
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**Background and aims:** To investigate the role of Rosiglitazone in high glucose(HG)-induced monocyte chemoattractant protein-1(MCP-1) expression by rat mesangial cells.
**Materials and methods:** After resting in serum-free medium for 24 h,the rat mesangial cells were cultured in the medium with normal glucose (5.6 mM, NG); high glucose (25 mM, HG); HG+5 μM Rosiglitazone; HG+20 μM Rosiglitazone and HG+PDTC (a NF-κB inhibitor). MCP-1 mRNA expression were measured by semi-quantitative RT-PCR assay. Activation of nuclear factor-κB(NF-κB) of rat mesangial cells were measured by electrophoretic mobility shift assay (EMSA). The levels of MCP-1 in the supernatants were determined by enzyme-linked immunosorbant assay (ELISA.)
**Results:** Semi-quantitative PCR showed that high-glucose (450 mg/dl,HG) increased the ratio of PCR products for MCP-1 to housekeeping gene GAPDH on densitometric results at 24 h by 2.8-fold,which was prevented by Rosiglitazone (20μmol/L) pretreatment.The NF-κB binding activity was 2.5-fold higher in MCs exposed to HG compared to basal glucose ($P < 0.05$).When the MCs were cultured in the presence of Rosiglitazone (20μmol/L) for 1 h,there was a highly significant reduction in NF-κB binding activity ($0.8 \pm 0.2$ vs $2.5 \pm 0.3$ $p < 0.01$).
**Conclusion:** This study suggests that Rosiglitazone may inhibit high glucose-induced NF-κB activation and MCP-1 expression in mesangial cells. These findings may provide an experimental evidence for further evaluating the possibly protective effect of Rosiglitazone against diabetic nephropathy.

## 1262

**Serum paraoxonase activity and paraoxonase L55M and G192A polymorphisms in Type 2 diabetic patients: the relationship with microvascular complications**
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**Background and aims:** lipid disorders and oxidative stress are risk factors shared by macrovascular and diabetic microvascular disease. Oxidative stress can be sustained by metabolic derangement but also by reduced defence. Paraoxonase (PON), an HDL-associated esterase, prevents the transformation of LDL into biologically active, oxidized, atherogenic particles. Its activity is affected by PON1 gene polymorphisms. Serum PON activity and the relationship of PON1 gene polymorphisms with microvascular complications of type 2 diabetes have been investigated in a cross-sectional study.
**Materials and methods:** the cohort included 106 control subjects and 317 type 2 diabetic patients. Serum PON activity was measured using paraoxon as a substrate. Two PON1 gene polymorphisms (L/M, Leu55Met and Q/R, Gln192Arg) were detected by the RFLP method (NlaIII and AlwI enzymes, respectively) after appropriate PCR.
**Results:** the mean PON activity did not differ in patients and controls ($66 \pm 41$ vs $58 \pm 44$ nmol/min/ml, $p=0.09$). The distributions of PON1 activity by genotypes were the same in both populations, i. e. for the PON1 L55M polymorphism LL>LM>MM and for the PON1 Q192R polymorphism RR>RQ>QQ (Anova oneway, $p<0.0001$). In both controls and diabetics, the two polymorphisms exert addictive effects on PON activity so that the lowest values were detected in homozygous for both M and Q alleles while the highest levels for homozygous for both L and R alleles ($p=0.009$ for controls, $p=0.0008$ for diabetics). There were no differences in PON1 activity in the presence or absence of microvascular complications. In particular, superimposible values ($p=0.925$) were observed in normoalbuminurics (30% of the whole diabetic cohort; $69 \pm 41$), microalbuminurics (44%; $69 \pm 41$) and subjects with overt nephropathy (26%, $68 \pm 44$ nmol/min/ml). In type 2 diabetes the Q192 allele frequency was 0.66 (R192: 0.34), the frequency of the L55 allele was 0.57 (M55: 0.43), both not significantly different from those observed in the control group. Neither the PON1-55 nor the PON1-192 polymorphism were correlated with the presence and/or the severity of diabetic retinopathy. In the same way, the distribution of the QQ,

QR and RR genotypes of the Gln192Arg polymorphism was similar in type 2 diabetics with normoalbuminuria (42.5, 47.9 and 9.6%) when compared with microalbuminurics (41.5, 50.0 and 8.5%) and those with overt nephropathy (42.2, 50.0 and 7.8%; p=0.98). On the contrary, the LL genotype was significantly more common both in patients with microalbuminuria (25.5%) and in those with overt nephropathy (24.6%) compared with those with normoalbuminuria (12.7%; p=0.03). Multiple logistic regression analysis shows that systolic blood pressure (p=0.04), coexistance of retinopathy (p=0.05) mainly the more advanced form of proliferative retinopathy (p=0.019), HbA1 c (0.012) and the LL genotype (p=0.005) are independently associated with raised urinary albumin excretion in type 2 diabetes.
**Conclusion:** these data suggest that people with type 2 diabetes and LL genotype at position 55 of the PON1 gene are more susceptible to renal complications. On the other side, the LL genotype was not associated with retinopathy. In our study, no role on microvascular complications can be assigned either to the PON1 Gln192Arg polymorphism or to PON serum activity.

## 1263

**The polymorphism of manganese superoxide dismutase is associated with microvascular complications in Korean Type 2 diabetic patients**
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**Background and aims:** Manganese superoxide dismutase (Mn-SOD), which is the essential enzyme for scavenging the reactive oxygen species in mitochondria, plays an important role in preventing the diabetic microvascular complications. It has been reported that a valine or alanine polymorphism at amino acid position 16 [Val(16)Ala] of Mn-SOD may be associated with diabetic microvascular complications in type 2 diabetic patients. We evaluated whether the Val(16)Ala polymorphism of Mn-SOD is associated with diabetic microvascular complications in Korean type 2 diabetic patients.
**Materials and methods:** A total of 178 non-diabetic control subjects (102 men and 76 women aged 51.3 ± 2.7 years) and a total of 371 type 2 diabetic patients (223 men and 148 women aged 53.1 ± 3.4 years) were enrolled from out-patients of Hallym University Medical Center, ChunCheon Sacred Heart Hospital. This study has been reviewed by the ethics committee and performed in accordance with the ethical standards laid down in the Helsinki Declaration. The stages of diabetic nephropathy, retinopathy and neuropathy were determined from urinary protein-creatinine ratio, fundus examination and clinical symptoms or signs respectively (non-nephropathy, microalbuminuria, overt proteinuria; non-retinopathy, non-proliferative, proliferative; non-neuropathy, mild, advanced). Genomic DNA was extracted from peripheral blood cells and genotyping of Val(16)Ala of Mn-SOD was done by PCR-RFLP with a restriction enzyme, *Bsaw* I.
**Results:** The allele frequency and the genotype distribution were not significantly different between the control subjects and diabetic patients [control subjects vs. diabetic patients; allele frequency (V/A), 0.879/0.121 vs. 0.896/0.104; genotype distribution (VV/VA+AA), 82.0/18.0 vs. 84.6/15.4]. The allele frequencies in both groups were consistent with the Hardy-Weinberg equilibrium. The frequency of the VA+AA genotype was significantly lower in the microalbuminuria and overt proteinuria groups compared with non-nephropathy group (non-nephropathy group 16.8%, microalbuminuria group 12.8%, overt proteinuria group 12.2%). While the frequency of the VA+AA genotype was not different according to the stages of retinopathy, the prevalence of VA+AA genotype was significantly more frequent in the non-neuropathy group than in the advanced neuropathy group (non-neuropathy group 16.7%, mild neuropathy group 16.9%, advanced neuropathy group 12.2%). When the relationships between the variables including Mn-SOD genotype and the progression of diabetic microvascular complications were analyzed by logistic regression, presence of hypertension, duration of diabetes and VA+AA genotype were independently associated with diabetic nephropathy and neuropathy in this study.
**Conclusion:** Our results demonstrate that the Val(16)Ala polymorphism of Mn-SOD may be independently associated with nephropathy and neuropathy in Korean type 2 diabetic patients but this polymorphism seems to be unrelated to the development of the type 2 diabetes in Korean population. Further large-scale cross-sectional prospective studies are needed to clarify the pathophysiological association between the polymorphism of Mn-SOD and the development and progression of diabetic microvascular complications in type 2 diabetic patients.

## 1264

**Effect of cigarette smoking on soluble adhesion molecules and their association with risk factors for microangiopathy**
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**Background and aims:** The development of microvascular complications is related to multiple risk factors including hyperglycaemia and hypertension. Cigarette smoking is a concomitant risk factor for microangiopathy. Plasma circulating adhesion molecules may be used as markers of endothelial dysfunction in diabetic patients and in several studies elevated levels have correlated with the development of microangiopathy. The effect of smoking on soluble adhesion molecules in type 1 diabetic patients has not been clarified. The aim of our study was to evaluate whether smoking had an adverse effect on plasma soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble intercellular adhesion molecule-1 (sICAM-1) and to analyse their relationship with metabolic control and ambulatory blood pressure, in a group of young type 1 normotensive normoalbuminuric diabetic patients.
**Materials and methods:** We studied 61 normotensive normoalbuminuric type 1 diabetic patients without chronic diabetic complications and with a diabetes duration between 5–15 years (44% men, 36% smokers, diabetes duration 9 ± 2 years, age 30 ± 6 years, albumin excretion rate 9,9 ± 6 mg / 24 h) and 32 normotensive controls (50% men, 40% smokers, age 30 ± 5 years). In all patients ambulatory blood pressure monitoring (ABPM) was performed using an oscillometric recorder (Spacelabs 90207) and albumin excretion rate was measured in two 24 h samples by immunoturbidimetry. Plasma concentration levels of sVCAM-1 and sICAM-1 were determined in all participants. In order to evaluate the acute effect of cigarette exposure we measured plasma sVCAM-1 and sICAM-1 before, immediately after and at 10 minutes after smoking two cigarettes in the 22 diabetic and 13 control subjects that were current smokers.
**Results:** The ABPM confirmed that all subjects were normotensive according to the British Hypertension Society recommendations. ABPM was similar in diabetic patients and control subjects except for nocturnal systolic BP (106 ± 8 vs 101 ± 8, p=0,022). No differences were found in BP between smokers and non-smokers. $HbA_{1c}$ was higher in diabetic patients that smoked than in non-smokers (7,7 ± 1 vs 7,0 ± 1, p=0,029). sVCAM-1 levels were higher in type 1 diabetic smokers than in control smokers (426 ± 135 vs 338 ± 73 ng/ml, p=0,043). sICAM-1 levels were higher in smokers than in non-smokers when the whole population (diabetic and control subjects) was considered (280 ± 74 vs 233 ± 46, p=0,001) and when type 1 diabetic smokers and diabetic non-smokers were compared (286 ± 72 vs 233 ± 51, p=0,008). We found no significant changes after smoking two cigarettes in sVCAM-1 and sICAM-1 concentrations. In diabetic patients sICAM-1 correlated positively with $HbA_{1c}$ (r=0,34, p=0,027), diurnal mean BP (r=0,41, p=0,018) and diurnal sistolic BP (r=0,34, p=0,030). These correlations remained significant after adjusting for age, gender, body mass index and smoking.
**Conclusions:** In type 1 diabetic patients without complications smoking is associated with higher levels of soluble adhesion molecules. Soluble adhesion molecules correlated with known risk factors for the development of microangiopathy suggesting that they may be early markers of endothelial dysfunction before clinical evidence of complications.

## 1265

**Sustained prevention of albuminuria but not nephromegaly 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 still a matter of debate. In this study we tested the effects of Rosiglitazone (R), a PPAR-γ agonist and potent insulin sensitizer, on the development of albuminuria and nephromegaly, in streptozotocin-induced diabetes in Sprague-Dawley rats and examined some of the mechanisms involved.
**Materials and methods:** Four groups of 6 Sprague Dawley rats of 150–200 g body weight (b.wt.), 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 b.wt. and confirmed 48 h later by a blood glucose level higher than 22 mM. Diabetic animals were treated daily with subcutaneous Insulatard, 0.01 U/g b.wt., adjusted to maintain glycaemia at around 25 mM in both groups of diabetic animals. R was administered as a pow-

der mixed in the food at an approximate dose of 0.005 mg/g b.wt./day after adjustment for animal weight and food consumption. D and DR animals were pair fed. Body weight and blood glucose (one Touch glucometer) were measured weekly. Blood pressure (tail cuff non-invasive method) was monitored monthly. Animals were sacrificed at 1, 3, and 9 months. Albuminuria was determined in the last week of each study period (Exocell-Nephrat Albuminuria kit) in animals kept in individual metabolic cages for 24 h urine collection. At necroscopy, wet left kidney weight was measured after removal of perirenal adipose tissue and of the renal capsule. Kidney tissue was stored for further investigations on cortical macrophages infiltration.

**Results:** Body weight was lower at 9 months in D (mean±SD, 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 (mean±SD, 30 ± 5.2 mM) and DR (29 ± 3.5 mM). Systolic blood pressure was not statistically different in D (mean±SD, 167 ± 7 mmHg) versus DR (158 ± 4) and in C (156 ± 8) versus CR (147 ± 12), though both control and diabetic R treated groups had numerically lower values. Albuminuria did not differ after 1 and 3 months of diabetes among the four groups. By 9 months there was a significant increase in albuminuria in the diabetic rats (D, median [interquartile range], 78 mg/24 h [46.4–158.4]) versus controls (C, 14 mg/24 h [4.1–25.4]; $p<0.05$), which was totally prevented by R (DR, 6 mg/24 h [2–14]; $p<0.05$ versus D, ns versus C). R had no effect on albuminuria in control animals. This was paralleled by a significant up to 60% inhibition of macrophage infiltration in the glomeruli and peritubular interstitium of DR versus D ($p<0.05$). No changes were seen in C vs CR. Kidney weight expressed as ratio of b.wt. (mg/g) was higher in D (mean±SD, 3.3 ± 0.15 mg/g) versus C (2.3 ± 0.28) at 3 months ($p<0.001$); this difference persisted at 9 months. R did not affect diabetes-induced kidney growth at 3 months (3.2 ± 0.7) and 9 months (3.5 ± 0.14). There was also no effect of R in control animals.

**Conclusion:** Rosiglitazone prevents the development of albuminuria in streptozotocin-diabetic rats but has no effect on nephromegaly. These effects are independent of blood glucose control and blood pressure levels. Prevention of albuminuria may be mediated by R-induced anti-inflammatory effects.

*Supported by: GlaxoSmithKline*

## 1266

**Skin microcirculation in the upper and lower extremities of diabetic patients with and without autonomic neuropathy**

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**Background and aims:** In contrast to lower extremities, upper extremities are only exceptionally affected by diabetic gangrene. Neuropathy and impaired blood supply, resulting either from macro- or microangiopathy, or both, are the most important risk factors for ulceration and gangrene. Microvascular blood flow in human skin is subject to rhythmic variations reflecting the influence of heartbeat, respiration, intrinsic myogenic activity, neurogenic factors and endothelial activity. The aim of our study was to test the hypothesis that basal skin blood flow (BSBF) and its dynamic components differ 1) among diabetic patients without autonomic neuropathy and with it and healthy control subjects, and 2) among the upper and lower extremities.

**Patients and methods:** Basal skin blood flow (BSBF) at 4 recording sites with predominantly nutritive capillary circulation (right and left caput ulnae, right and left medial malleolus) was measured by laser Doppler flowmetry in 38 diabetic patients without cardiovascular autonomic neuropathy (D), 31 neuropathic diabetic patients (DAN) and 78 healthy controls (C). Wavelet transform was applied to the laser Doppler signal.

**Results:** The results are summarized in Table 1 and 2. In absolute terms, mean flow, mean amplitude of the total spectrum and mean amplitudes at all frequency intervals were lowest in D, followed by DAN and highest in C at all recording sites except for the right leg where higher values were observed in DAN than in C. The differences were statistically significant only in the left but not in the right extremities. Within all three groups, mean flow and spectral amplitudes were significantly higher in the arms than in the legs, besides there was a significant difference between the two arms in D.

**Conclusion:** Decreased BSBF in D in comparison with C and DAN indicates that microangiopathy precedes autonomic neuropathy. The differences between the upper and lower extremities provide a possible explanation for the higher prevalence of ulceration and gangrene on the lower extremities in comparison to the upper. The difference between the two arms which was found in D, points to an uneven progression of autonomic neuropathy and allows for speculation that the left arm is the latest to be affected.

Mean flow at the four recording sites (Wilcoxon T - test).

| Recording site/Group | F1 (r arm) | P F1/F2 | F2 (r leg) | P F1/F3 | F3 (l arm) | P F3/F4 | F4 (l leg) | P F2/F4 |
|---|---|---|---|---|---|---|---|---|
| D | 36,36 ± 35,40 | **0,000** | 17,55 ± 11,48 | **0,003** | 25,77 ± 18,12 | **0,001** | 16,75 ± 15,14 | ns |
| DAN | 36,28 ± 22,0 | **0,000** | 18,60 ± 10,84 | ns | 39,40 ± 31,50 | **0,000** | 16,39 ± 6,29 | ns |
| C | 45,40 ± 34,95 | **0,000** | 23,60 ± 19,47 | ns | 48,62 ± 38,52 | **0,000** | 24,77 ± 17,15 | ns |

Mean flow and mean amplitude of total spectrum in the three groups

| | Kruskall - Wallis test D/DAN/C | Mann - Whitney test D/C | Mann - Whitney test DAN/C | Mann - Whitney test DAN/D |
|---|---|---|---|---|
| F1 - right arm Mean flow | ns | ns | ns | ns |
| F1 - Mean amplitude of total spectrum | ns | ns | ns | ns |
| F2 - right leg Mean flow | ns | ns | ns | ns |
| F2 - Mean amplitude of total spectrum | ns | ns | ns | ns |
| F3 - left arm Mean flow | 0,000 | 0,000 | ns | ns |
| F3 - Mean amplitude of total spectrum | 0,000 | 0,000 | ns | 0,009 |
| F4 - left leg Mean flow | 0,005 | 0,004 | 0,025 | ns |
| F4 - Mean amplitude of total spectrum | 0,040 | 0,017 | ns | ns |

## 1267

**Functional abnormalities of the skin micro-circulation in diabetes mellitus Type 1 as revealed by a combined laser-Doppler and pharmacological studies**

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**Background and aims:** The very early functional indices of the diabetic microangiopathy are not clearly determined. More information in this area may have, however, important clinical significance. Therefore the study of the skin microcirculation with laser-Doppler flowmetry combined with pharmacological testing of the basic and reactive flow was undertaken.

**Materials and methods:** Values of the skin microcirculatory blood flow using the laser-Doppler flowmetry were determined in the form of specific pharmacological testing – before and during methacholine (MCh, endothelial factor) and nitroglycerin (NTG, extra-endothelial factor) intravenous infusion. Such studies were performed in 3 groups of subjects: A – type 1 diabetes mellitus of less than 5 years duration – 16 subjects, B – more than 5 year duration and with retinopathy – 10 subjects, and C – control group – 20 subjects. The maximal reached perfusion as well as the mean values from 2, 5 and 10 minutes of test were estimated.

**Results:** In the methacholine test assessing endothelium function the lowest relative increase of maximal perfusion was observed in group B 7,76 (CI95% 5,05–9,56), vs. group A 10,95 (CI95% 5,18–18,49) and group C 13,43 (CI95% 8,31–24,92). The analysis of relative mean perfusion growth showed lowest values in group B, and highest in group C:

| | MCh – 2 min | MCh – 5 min | MCh – 10 min |
|---|---|---|---|
| Group A | 4,27 (CI95% 1,46–10,50) | 2,74 (CI95% 1,28–5,46) | 2,15 (CI95% 1,12–3,97) |
| Group B | 2,38 (CI95% 0,99–3,52) | 2,03 (CI95% 1,16–2,60) | 1,74 (CI95% 1,03–2,46) |
| Group C | 4,91 (CI95% 1,48–8,47) | 3,87 (CI95% 1,40–7,11) | 2,88 (CI95% 1,24–6,25) |

The nitroglycerin test showed the lowest microvessel reactivity in group B – the maximal relative increase 2,58 (CI95% 1,88–3,67), vs. group A – 3,97 (CI95% 2,30–7,59) and group C – 4,12 (CI95% 1,81–7,17). The analysis of time compartments of the test showed similar relations:

| | NTG – 2 min | NTG – 5 min | NTG – 10 min |
|---|---|---|---|
| Group A | 1,39 (CI95% 1,07–2,08) | 1,38 (CI95% 1,01–2,12) | 1,38 (CI95% 1,03–1,92) |
| Group B | 1,01 (CI95% 0,74–1,26) | 1,05 (CI95% 0,77–1,38) | 1,07 (CI95% 0,75–1,35) |
| Group C | 1,31 (CI95% 0,92–2,35) | 1,46 (CI95% 1,00–2,42) | 1,37 (CI95% 1,03–2,25) |

**Conclusion:** The reactive increase of the skin microcirculatory flow both after MCh and after NTG was significantly lower in diabetes mellitus type 1 groups (in group B more than in group A) in comparison with control. This finding points to the existence of the very early functional microcirculatory abnormalities as revealed by laser-Doppler and pharmacological testing. The proposed method is efficient and safe.

# PS 133

# Glycation and oxidative stress

## 1268

**The structural and functional analysis of the human glyoxalase-1 gene**

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**Background and aims:** Advanced glycation endproducts (AGEs) accumulate at an accelerated rate in diabetes mellitus and induce change in the vascular endothelium that may contribute to micro- and macrovascular disease. Methylglyoxal (MG), which forms MG-derived AGEs the primary intracellular AGE induced by hyperglycaemia, is elevated in subjects with diabetes and vascular disease. Detoxification of MG occurs through the glyoxalase system incorporating glyoxalase-1 and glyoxalase-2. Perturbations of the glyoxalase gene (GLO1) may result in vunerability to vascular complications through alterations in AGE interactions. We used laboratory-based methods and computational techniques to investigate the structure and function of GLO1.
**Materials and methods:** Exons were predicted bioinformatically and confirmed by PCR. Exons, 3'UTR and 4.5 kb of 5' flanking region were screened by DHPLC and searched at SNP databases for polymorphisms. SIFT and BLOSSUM62 matrix predicted tolerance and functionality of coding variants. BLAST and polyA prediction tools and RLM-RACE were used to determine alternative 3' and 5'UTRs. AceView and PCR were used to analyse alternative splice variants. SAGE and RT-PCR was used to assess human and murine temporal and spatial expression. Promoter prediction tools and the creation of 5' nested deletions assessed promoter function in pGL3-basic under conditions of hyperglycaemia. Comparative genomics was determined computationally.
**Results:** 6 GLO1 exons were identified. 70 SNPs were identified bioinformatically and 17 by DHPLC. 2 amino acid substitutions were identified (Ala 111 Glu, Arg 123 Gln), predicted to be tolerant and deleterious and had A allele frequencies of 48% and <1% respectively. A common SNP was found in the Kozak sequence (-7 T to C, allele frequency =31%). There were no alternative splice varitants, 4 alternative transcription start sites and 83 3' UTRs were identified. Conserved regulatory regions were predicted 5' to the transcription start site, in the distal promoter. Many predicted conserved transcription regulatory elements were suggested in the 5'UTR. Areas of conservation were predicted in peri-coding regions. Reporter gene expression was repressed from –207 to –794 and enhancer elements identified to position –1024. Thereafter, repressed expression occurred to position –2483, followed by enhanced expression to –3203 and with a 50% reduction in reporter expression to position –4494. No significant regulatory responce occurred after 48 hours of 20 mM glucose incubation.
**Conclusion:** GLO1 demonstrates multiple sequence variants at DNA and mRNA levels, is a highly regulated gene with key areas of sequence conservation and has SNPs that are predicted to affect function. A differential ability of glyoxalase-1 to reduce the formation and subequent interaction of AGEs may have a role in the structural and functional manifestations of diabetic vascular disease.
*Supported by: Medical Research Council*

## 1269

**Serum levels of hydroimidazolone are increased in retinopathy of Type 2 diabetes**

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**Background and aims:** Advanced Glycation End products (AGEs) are thought to play a role in the pathogenesis of diabetic retinopathy. However, it is not known which specific AGE(s) being most closely linked to retinopathy. Methylglyoxal is a highly reactive aldehyde in vivo and can form hydroimidazolone, an AGE formed by protein modification. We wanted to study the possible role of hydroimidazolone in diabetic retinopathy.
**Materials and methods:** We cross-sectionally studied the relationship between serum levels of hydroimidazolone and retinopathy in 237 type 2 diabetic patients using a competitive immunoassay with monoclonal anti-

bodies against hydroimidazolone ( Kilhovd et al, Metabolism. 2003 Feb;52(2):163–7 ). Retinopathy was graded ad modum ETDRS from seven standard field stereo photographs. The subjects had a mean age of 66 years ( range 21–92 years ) and consisted of 132 males and 105 females.
**Results:** Hydroimidazolone was significantly increased in both non-proliferative, 4.60 (2.49–74.00) U/ml, median (min–max), and proliferative retinopathy, 4.88 (2.50–24.24) U/ml compared to patients without retinopathy 4.03 (2.13–24.57) U/ml, p=0.007 and p=0.003 respectively, although patients with clearly elevated p-creatinine (>200 µmol/l) were excluded. We also investigated whether hydroimidazolone could be of possible importance for initiation of retinopathy by studying subjects with a duration of diabetes below the median of 14 years. Serum levels of hydroimidazolone in this subgroup, too, were significantly higher in subjects with retinopathy, 4.72 (2.50–12.47) compared with those without retinopathy, 3.94 (2.13 – 24.57) p=0.006. Hydroimidazolone and HbA1c were not correlated (r = 0.04).
**Conclusion:** Serum hydroimidazolone is strongly associated with diabetic retinopathy. As it also is increased in those with early onset of retinopathy, this may suggest a pathogenic role of hydroimidazolone in diabetic retinopathy.

## 1270

### Time course of specific AGEs during optimized glycaemic control in Type 2 diabetes

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**Background and aims:** Several AGE compounds are formed in the hyperglycaemic state. Although serum AGEs correlate with average glycaemic control and predict the development of complications, it is not known how serum AGEs change during optimization of diabetes therapy.
**Results:** We evaluated the change in serum levels of CML ($N^{\varepsilon}$-carboxymethyllysine) and MGHI (Methylglyoxal-derived hydroimidazolone), as well as markers of endothelial function in 28 subjects with type 2 diabetes, who were poorly controlled on oral agents, before and after the institution of insulin therapy. Their mean age (±SEM) was 58 ± 2 years, body mass index 27.7 ± 0.8 kg/m$^2$, and known duration of diabetes was 8.1 ± 0.9 years. With insulin treatment fasting blood glucose levels dropped from 12.1 ± 0.9 mmol/l to 6.9 ± 0.3 and 8.1 ± 0.4 mmol/l after 3 and 7 months, respectively (both p<0.001), while $HbA_{1c}$ decreased from 10.0 ± 0.3 to 7.8 ± 0.2% (p<0.001). Endothelial function improved as indicated by a small but significant decrease of sICAM-1 (152 ± 10 to 143 ± 8 ng/ml, p<0.02) and sE-selectin (111 ± 16 to 102 ± 12 ng/ml, p<0.02) levels. In contrast, we observed only a tendency to a decrease in CML levels (110 ± 22 to 86 ± 13 µg/mg protein, p=0.086), but a small increase of MGHI (from 0.23 ± 0.02 to 0.29 ± 0.04 U/mg protein, p<0.02). At baseline, 16 patients used metformin, and they had similar levels of CML and MGHI as the 12 non-metformin users, although their $HbA_{1c}$ was lower (9.4 vs. 10.7%). During insulin, patients receiving concomitant metformin therapy tended to have lower MGHI levels as patients receiving no metformin therapy (0.18 ± 0.03 vs. 0.32 ± 0.04 U/mg protein, p=0.11).
**Conclusion:** We conclude that, although insulin therapy improved $HbA_{1c}$ and markers of endothelial function, the levels of serum AGEs did not follow the same time course. This suggests that these specific AGEs are influenced by other factors in addition to overall glycaemia, like oxidative stress.

## 1271

### Efficacy of antioxidant therapy in suppression of poly(ADP-ribose)polymerase activation in the brain of rats with experimental diabetes

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**Background and aims:** Enhanced oxidative stress is well-recognized mechanism in the pathogenesis of diabetic neuropathy. Recent findings from our group indicate activation of brain poly(ADP-ribose)polymerase (PARP) that may be considered as downstream effector of oxidative injury. To establish a causal relation between diabetes-associated pro-oxidative state and poly-ADP-ribosylation in brain, we evaluated effects of free oxygen radical scavengers such as alpha-tocopherol, nicotinamide and alpha-lipoic acid applied in doses to achieve a similar antioxidant effects on poly(ADP-ribose)polymerase activity.
**Materials and methods:** After 4 weeks of STZ-induced diabetes (70 mg/kg of body weigt, i.p.), rats were treated for 2 weeks with or without nicotinamide (NAm, 100 mg/kg, i.p.), alpha-tocopherol (alpha-T, 15 mg/kg, per os) or alpha-lipoic acid (LA, 40 mg/kg, i.p.). PARP activity was measured by incorporation of labeled ADP-ribose from [U-$^{14}$C]$NAD^+$ into brain nuclei proteins. Degree of DNA damage was determined spectrophotometrically. Intermediates of glucose metabolism, $NAD^+$ and contents of reduced glutathione (GSH) were assayed enzymatically in protein- and ion-free perchloric acid extracts of brain.
**Results:** Diabetes caused 23.5 ± 2.9 % (p<0.05) reduction in the level of such protective endogenous antioxidant as GSH in diabetes vs control, that was accompanied by three-fold accumulation of thiobarbituric acid reactive substances thus indicating impaired cellular scavenging activity against oxidative stress. It was shown that previously defined 21.0 ± 1.8 % (p<0.05) elevation of PARP activity is presumably related to DNA damage, obligatory stimulus for the activation of the enzyme, since a distinct 4.9-fold increase in output of acid soluble low-molecular fragments from DNA was established as compared to control (p<0.05). All antioxidants approximately to the same extent arrested accumulation of lipid peroxidation products and partially restored GSH contents (p<0.05). Nevertheless, they elicited dissimilar ability to affect PARP, DNA integrity and some indices of cell metabolism. NAm administration showed the most significant down-regulation of PARP activity to 3.1 ± 0.4 vs 4.3 ± 0.5 mmol $10^{-8}$of bound radioactivity $min^{-1}$/mg protein in diabetes, p<0.05, whereas LA and to a less extent alpha-T attenuated it to 3.4 ± 0.3 and 3.8 ± 0.3 respectively, p<0.05. The degree of DNA damage markedly decreased in case of NAm and LA treatments and only slightly was counteracted in alpha-T-treated group (p<0.05). More substantial action of NAm against diabetes-induced abnormalities as opposed to LA corresponded to its much greater normalizing effects on blood glucose, brain sorbitol, decreased $NAD^+$ levels and free cytosol $NAD^+$/NADH ratios. No significant differences between alpha-T treatment and diabetes were noted with respect to sorbitol content and redox state of free cytosol NAD(H)-couples.
**Conclusion:** The data suggest that brain dysfunction in early diabetes is associated with a propagation of oxidative stress which may be responsible for DNA damage-mediated activation of PARP. More efficient role of water-soluble vitamins, especially NAm, in down-regulation of PARP seems likely to be contributed to the wider range of metabolic and signaling events that can be influenced. In particular NAm case, assessment of its direct inhibiting action on PARP may deserve further investigation.

## 1272

### Role of activation of NF-κB and AP-1 by oxidative stress in atherosclerosis of diabetic patients

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**Background:** The aim of this study was to evaluate the possible role of oxidative stress in atherosclerosis of diabetic patients by measuring intracellular ROS generation and the activation of transcription factors, including nuclear factor-kappa B (NF-κB), activator protein-1 (AP-1) in isolated peripheral blood mononuclear cells (PBMCs).
**Methods:** 66 patients matched for $HbA_{1C}$ (10.6 ± 2.7%) with diabetes mellitus (28 males, 38 females; age 56.1 ± 13.4 years; duration of diabetes 115.7 ± 83.4 months) were selected for this study. Patients with DM included in this study were divided into DM patients with carotid normal intima-media thickness (Group II) and DM patients with increased intima-media thickness (Group III). We selected at random 57 healthy controls matched for age and sex with DM patients (Group I). Dichlorodifluorescein (DCF)-sensitive intracellular ROS was measured by fluorescent spectrometry. The activities of NF-κB, AP-1 in PBMCs were measured by electrophoretic mobility shift assay. The amount of TGF-$\beta_1$ present in PBMC lysate was determined by quantitative sandwich enzyme immunoassay.
**Results:** Spontaneous and $H_2O_2$ (or phorbol-12-myristate-13-acetate, PMA) stimulated ROS were significantly higher in PBMCs from patients with Group III than in those Group II, and these were also higher in Group I. No difference between the groups in terms of sex, age, BMI, blood pressure, total cholesterol, triglyceride and HDL-cholesterol were evident. Group II had an 1.33 fold higher AP-1 activity but similar NF-κB activity. On the other hand, Group III had 2.64 fold higher NF-κB activity, a 1.79 fold higher AP-1 activity than Group I. Moreover, the activities of NF-κB and AP-1 were significantly higher in Group III than in Group II. The TGF-$\beta_1$ protein in PBMCs was higher in Group III than Group II (3.11 ± 0.39 vs 1.78 ± 0.68). No difference in TGF-$\beta_1$ protein was observed in PBMCs of Groups I and II.

The levels of TGF-$\beta_1$ were significantly correlated with NF-κB and AP-1 activity in PBMCs and in carotid artery IMT.
**Conclusion:** The present study demonstrates that intracellular ROS generation, and NF-κB and AP-1 activation in PBMCs strongly correlate with carotid artery IMT and TGF-$\beta_1$ expression in PBMCs. These clinical results suggest that increased oxidative stress in PBMCs may play a role in the pathogenesis of atherosclerosis in DM patients.

## 1273

**S 21403, a new short acting insulin secretagogue, improves the oxidative stress induced by post-prandial hyperglycaemia in Type 2 diabetic patients**

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**Background and aim:** Oxidative stress and inflammation are involved in the pathogenesis of diabetic complications and has been shown to be increased in type 2 diabetic patients. Hyperglycaemia, particularly the acute rise in glucose concentration during the post-prandial phase, is a major component of the oxidative stress. The aim of this study was to assess the acute effect of a new insulin secretagogue S 21403 vs placebo on oxidative stress and inflammation induced by hyperglycaemic spikes following meals in type 2 diabetic patients.
**Material and method:** A total of 40 type 2 diabetic out-patients were included in a monocentric double-blind, cross-over study to receive in a randomised order a single dose of S 21403 (40 mg) or placebo after a 7 day placebo run-in period.
The plasma concentrations of nitrotyrosine, malondialdehyde (MDA), interleukin-6 (IL-6), interleukin-18 (IL-18), TNF-α, total radical-trapping antioxidant parameter (TRAP) and oxidized LDL were assessed over 120 min during a breakfast-test. The changes between T0 and T120 min were measured for all parameters.
The difference between treatments (S 21403 versus Placebo) and its associated standard error were estimated performing a model of analysis of variance taking into account the cross-over design and compared using a two-sided Student t-test statistic with a 5% type I error. The results are expressed as mean ± SD.
**Results:** 40 type 2 diabetic patients aged of 59 ± 8 years with an HbA1 c of 6.9 ± 0.5 %, a BMI of 28 ± 3 kg/m$^2$, a diabetes duration of 57 ± 43 months, previously treated either by diet alone (31 patients, 77.5%) or sulfonylurea (9 patients, 22.5%) completed the study. The rapid stimulation of insulin secretion obtained with S 21403 induced a significant decrease in the post-prandial plasma glucose level compared to placebo. Nitrotyrosine, MDA, IL-6, IL-18, TNF-α and oxidized LDL significantly decreased with S 21403 compared to placebo. The TRAP decrease was significantly lower with S 21403 compared to placebo, as described in the following table.

| | Changes between T0 and T 120min | | | |
|---|---|---|---|---|
| | S 21403 mean± SD (n= 40) | placebo mean ± SD (n= 40) | Estimate (SE) of the difference between treatment groups | p-value |
| Insulin (pmol/L) | 500 ± 315 | 321 ± 314 | 179 (44) | <0.001 |
| Glucose (mmol/L) | 0.7 ± 2.7 | 3.8 ± 2.3 | - 3.1 (0.3) | < 0.001 |
| Nitrotyrosine (μmol/L) | 0.05 ± 0.06 | 0.14 ± 0.07 | - 0.09 (0.01) | <0.001 |
| MDA (μmol/L) | 0.22 ± 0.33 | 0.38 ± 0.34 | - 0.16 (0.07) | < 0.05 |
| IL-6 (pg/ml) | 0.04 ± 0.13 | 0.18 ± 0.19 | - 0.14 (0.04) | < 0.001 |
| IL-18 (pg/ml) | 76 ± 159 | 205 ± 182 | - 129 (30) | < 0.001 |
| TNF-α (pg/ml) | 0.38 ± 0.99 | 1.57 ± 1.18 | - 1.19 (0.25) | < 0.001 |
| Oxidized LDL (UI/L) | 7.4 ± 10.0 | 16.0 ± 9.4 | - 8.6 (1.6) | <0.001 |
| TRAP (μmol/L) | -12.5 ± 11.1 | -16.9 ± 7.9 | 4.4 (1.8) | < 0.05 |

**Conclusion:** In type 2 diabetic patients, S 21403 significantly reduces oxidative stress and inflammation induced by acute hyperglycaemia during a meal-test, by decreasing the post-prandial hyperglycaemia through a rapid stimulation of insulin secretion. The management of post-prandial hyperglycaemia could contribute to the prevention of cardiovascular complications in patients with type 2 diabetes.
*Supported by: Servier*

# PS 134

# Regulatory effects on endothelial cells

## 1274

**High glucose reduces human endothelial progenitor cells differentiation to endothelial cells**

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**Background and aims:** Endothelial dysfunction (ED) is an important contributor of atherosclerosis. Recent studies suggest that ED is in part due to reduced angiogenic activity of mature endothelial cells and impaired vasculogenic potential of Endothelial Progenitors Cells (EPCs). In diabetic patients both insulin resistance and glucotoxicity are associated to endothelial dysfunction and atherosclerosis progression. Hyperglycemia induces ED through several mechanisms including hexosamine toxicity, DAG-PKC pathway and NF-kB hyperactivation, all leading to increased anion superoxide activity. To address the role of glucose toxicity on ability of EPCs to differenziate in mature endothelial cells , human EPCs were isolated from peripheral blood of healthy volunteers and seeded on fibronectin-coated plates in the presence or absence of high glucose (HG, 33 mM), high glucose and Benfotiamine (HG+BFT, 50 microM), a thiamine derivative able to bypass anion superoxide effect and induce glycolysis.
**Materials and methods:** Cells were mantained in the various treatment for 7 days. At the end, EPCs ability to form endothelial colonies (CFU) was assessed through double immunofluorescence. EPCs were identified by double positivity to acLDL DIL-conjugated and FITC-Lectin. Furthermore, we assessed expression of mature endothelial cells markers as CD31 / PECAM and FLK1/VEGFR2.
**Results:** Results suggest that HG reduces EPCs differentation in mature endothelial cells, when conpared with control culture. In particular, we observed: a) a reduced number of CFU, marked by double positivity to acLDL DIL-conjugated and FITC-Lectin, (HG 48% of control); b) a reduced number of CD31/PECAM positive cells (77% of control); c) a reduced number of FLK1/VEGFR2 positive cells (56% of control). Treatment with BFT recovers the effect of HG in all the parameters evaluated; in fact we observed: a) BFT increased number of CFU, marked by double positivity to acLDL DIL-conjugated and FITC-Lectin, (101% of control); b) BFT increased number of CD31/PECAM positive cells (110% of control); c) BFT increased number of FLK1/VEGFR2 positive cells (92% of control). Akt phosphorylation on Ser473 was markedly decreased under HG compared with control. Interestingly, treatment with BFT abolishes negative effects of HG on Akt phosphorylation.
**Conclusion:** These results suggest that glucotoxicity impairs EPCs ability to differenziate and proliferate in mature endothelial cells, thus contributing to progressive loss of endothelial function, typical of diabetic patients. Moreover, Benfotiamine is able to inhibit glucotoxicity effect, probably bypassing superoxide anion negative effect.
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## 1275

**Linoleic acid increases lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) expression in human aortic endothelial cells**

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**Background :** There is evidence that linoleic acid (LA) is a pro-oxidative and pro-inflammatory molecule that can induce endothelial dysfunction (ED). Patients with type 2 diabetes show increased concentrations of LA in all low density lipoprotein (LDL) subfractions and this alteration may contribute to ED in diabetes. Endothelial lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is the major receptor for oxidized LDL (oxLDL) and oxLDL uptake through this receptor induces ED. Endothelial LOX-1 expression is increased in diabetic rats and upregulated in response to high glucose and advanced glycation end products.
**Aim :** The aim of this study was to examine the role of LA in the dysregulation of endothelial LOX-1 in diabetes.
**Materials and methods :** Human aortic endothelial cells (HAECs) were incubated with LA (0.05–0.4 mM) for 6 to 48 hours. In some experiments, HAECs were preincubated with the antioxidants, N-acetylcysteine and vitamin C, the protein kinase C inhibitor, calphostin C, the activated protein-1 (AP-1) inhibitor, curcumin or the nuclear factor-κB (NF-κB) inhibitor, BAY11-7085. At the end of these incubation periods, LOX-1 protein and gene expression were measured by Western blot and PCR analysis, respectively.
**Results :** LA increased, in a dose-dependent manner, endothelial LOX-1 protein expression. Maximal effect was observed at 24 hours with 0.2 mM of linoleic acid (LOX-1 protein levels [% increase over control values] 196 ± 13, $P<0.001$). Preexposure of HAECs to antioxidants, calphostin C, curcumin or BAY11-7085 inhibited the stimulatory effect of LA on LOX-1 protein expression. LA also increased LOX-1 gene expression, with maximal effect occuring at 15 hours (LOX-1 mRNA expression [% increased over control values] 160 ± 19, $P<0.05$).
**Conclusion :** These results demonstrate that LA increases endothelial LOX-1 expression through oxidative stress-sensitive and PKC-dependent pathways. This effect seems to be exerted at the transcriptional level and to involve the activation of AP-1 and NF-kB. Upregulation of LOX-1 by LA may contribute to ED associated with type 2 diabetes.

## 1276

**Evidence for a role of selenium in down-regulation of P38 mitogen-activated protein kinase (P38MAPK) and cyclooxygenase-2 (COX-2) in human umbilical vein endothelial cells**

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**Background and aims:** Diabetes is associated with a significantly increased prevalence of cardiovascular complications including atherosclerosis. Essential pathogenic factors of diabetic atherosclerosis may include hyperglycemia and its toxication, insulin resistance and oxidative stress. The present study is designed for the investigation of the relationship between P38 mitogen-activated protein kinase (*P38MAPK*) and cyclooxygenase-2 (*COX-2*) and the role of Selenium in the prevention of diabetic atherosclerosis.
**Material and methods:** We initially investigated protein expression of P38 mitogen-activated protein kinase (*P38MAPK*) and cyclooxygenase-2 (*COX-2*) in human umbilical vein endothelial cells (HUVECs), which were incubated respectively with 25 mM glucose, 100 μg/ml BSA-AGEs, 100 nM insulin and 100 μM $H_2O_2$. We also studied the relationship between *P38MAPK* and *COX-2* protein expression by using SB203580, a specific inhibitor of *P38MAPK*. We then evaluated the direct effects of Selenium on the regulation of *P38MAPK* and *COX-2* protein expression.
**Results:** Our results demonstrated that both *P38MAPK* and *COX-2* had significantly higher expression in HUVECs incubated with 25 mM glucose, 100 μg/ml BSA-AGEs, 100 nM insulin and 100 μM $H_2O_2$ respectively. COX-2 activity was significantly reduced when *P38MAPK* was inhibited by SB203580. Furthermore, both *P38MAPK* and *COX-2* protein expression were significantly decreased in HUVECs with Selenium treatment.
**Conclusion:** The present study provides additional evidence that both *P38MAPK* and *COX-2* are involved in development of diabetic atherosclerosis, and also suggests that Selenium may play a role in the prevention of diabetic atherosclerosis by down-regulation of both *P38MAPK* and *COX-2* expression.
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## 1277

**Cell type specific induction of apoptosis by free fatty acids in human vascular cells**

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**Background and aims:** Plasma concentrations of free fatty acids (FFAs) are elevated in obesity and diabetes and contribute to the development of premature atherosclerosis. Since stearic acid and polyunsaturated fatty acids (PUFAs), but not oleic acid, induce apoptosis in human umbilical vein endothelial cells (HUVECs), this study aims at evaluation of FFAs' proapoptotic activities and of the respective underlying mechanisms in vascular endothelial and smooth muscle cells.
**Materials and methods:** After exposure (24 h) of HUVECs, human aortic endothelial cells (HAECs) and human smooth muscle cells (HSMCs) ***i)*** to plasma with high (350 μmol/l) vs. low (50 μmol/l) FFA concentrations, ***ii)*** to a defined mixture (final concentration: 300–900 μmol/l) of palmitic-, stearic-, oleic-, linoleic-, and α-linolenic acid (2,6 : 1: 3,6 : 9 : 1, respectively),

or *iii)* to purified FFAs (100–300 µmol/l), apoptosis and protein expression were determined by DNA fragmentation assays and Western blot analyses, respectively.
**Results:** Incubation of HSMCs and HUVECs with plasma obtained from individuals with elevated plasma FFA concentrations increased apoptosis by 3.6-fold ($p<0.05$, n=4) and 4.2-fold ($p<0.001$, n=10), respectively, compared with intraindividually matched low plasma FFA samples. Exposure of HSMCs and HAECs to the defined FFA-mixture (900 µmol/l) increased apoptosis by 5.3-fold ($p<0.001$, n=5) and 11.8-fold (n=2), respectively. Incubation with purified FFAs revealed PUFAs (~ +550%), but not palmitic acid to induce apoptosis in HSMCs and endothelial cells. The proapoptotic activity of the saturated FFA, stearic acid, in HUVECs (+234%) was 3.4-fold that induced in HSMCs (+68%). In contrast, the monounsaturated FFA, oleic acid, exhibited a 20-fold higher proapoptotic action in HSMCs (+240%) compared with HUVECs (+12%). In HSMCs, HAECs and HUVECs, FFA-induced apoptosis was completely abolished by the caspase inhibitor zVAD.fmk (30 µmol/l).
In HUVECs (n=6), the PUFAs' proapoptotic activity correlated ($p<0.01$) with increased protein expression of *E2F-1* (r=0.89) and with that of E2F's activator *c-myc* (r=0.7). Additionally, endothelial apoptosis was associated with reduced expression of the base excision repair protein *XRCC1* (r= −0.78, $p<0.01$), wheras c-myc's antagonist *mad* was not affected.
**Conclusion:** High FFA-plasma and a defined FFA-mixture triggered apoptosis in human venous- and aortic endothelial cells, as well as in human smooth muscle cells. In HUVECs, PUFA-induced apoptosis related to upregulation of c-myc and E2F-1. The proapoptotic activity of PUFAs was similar in endothelial- and smooth muscle cells, whereas stearic- and oleic acid differently affected endothelial- and smooth muscle cell apoptosis. Since apoptosis of vascular cells (endothelial- and smooth muscle cells) crucially affect vascular functions (e.g. endothelial permeability, intimal thickening), these data could help to support dietary recommendations using vasoprotective fatty acids (e.g. oleic acid) in order to prevent FFA-mediated vascular dysfunction in metabolic disorders.

## 1278

**Subendothelial deposition of von Willebrand factor in vitro under conditions of high glucose, addition of insulin and factors of local microenvironment**

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**Background and aims:** Diabetes mellitus is associated with alterations in plasma von Willebrand factor (vWF) content and platelet function. It is noteworthy that pro-thrombogenic vWF, deposited in basal membrane, mediates the adhesion of platelets to subendothelium after vascular injuri. Recent investigation show that in vitro high glucose concentration stimulates secretion of vWF into the medium by cultured endothelial cells. In this study we measured the extracellular accumulation of vWF by human umbilical vein endothelial cells (HUVEC), cultured in the presence of main „diabetic milieu" constituents-high glucose and insulin, as well as under influence of the factors produced by adjacent vessel wall cells-human smooth muscle cells (SMC) and fibroblasts.
**Materials and methods:** 1st passage HUVEC were seeded into gelatin-coated wells ($30x10^3$ cells/$cm^2$) and cultured in modified DMEM (5.5 mM glucose, supplemented with additional vitamins, glutamine, 20 % FCS and designated as control medium) for 6–7 days. To simulate hyperglycemia, cells were exposed to 28 mM glucose. Insulin was added to a concentration of 8 µg/ml. Primary cultures of umbilical artery SMC and skin fibroblasts were initiated by explant method. Culture medium, conditioned for 3 days by SMC and fibroblasts in secondary culture (3–7 passages) was collected and added to HUVEC at 25 % of the total medium volume. To determine vWF in subendothelial matrix, HUVEC were treated with 0.1 M $NH_4OH$ and the remaining material was used as a solid phase in ELISA method with primary (anti-vWF) and secondary (peroxidase-conjugated) antibodies. The results were expressed as medians, relative to cultures incubated with control medium. Statistic analysis was carried on by non-parametric Van der Waerden criterion, $P<0.05$ was considered statistically significant.
**Results:** The substances under study did not cause detectable cytotoxicity in HUVEC culture. Deposited subendothelial vWF was visualized by light microscopy using DAB substrate for the peroxidase reaction. Positive vWF staining was revealed as fibrillar network. According to ELISA estimation, 28 mM glucose and 8 µg/ml insulin somewhat lowered the quantity of vWF in matrix, but this effect was insignificant (92 % and 85 % correspondingly for high glucose and insulin, $P>0.05$, n=10). In this relation, similar results were obtained when glycosylation of subendothelial matrix was tested by biotinylated lectins. Insulin, but not high glucose, reduced binding of concanavalin A to the matrix emerged after treatment of HUVEC monolayer with urea (70% relative to control, $P<0.02$, n=23). Medium, conditioned by skin fibroblasts and added alone or together with insulin to HUVEC, did not change vWF content of matrix (96–99 %). Conversely, SMC conditioned medium, supplemented with insulin, caused significant lowering in subendothelial deposition of vWF (72 %, $P<0.05$, n=24).
**Conclusion:** High glucose and insulin do not appreciably change extracellular deposition of vWF in vitro. Our data indicate that SMC may produce the factors which are involved in regulation of subendothelial vWF content. This points up the importance of local vascular microenvironment-"diabetic milieu" relationship in alteration of hemostasis

## 1279

**Resveratrol protect oxidized LDL-induced cytotoxicity and adhesion molecules expressions in endothelial cells**

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**Background and aims:** Interaction between oxidized LDL and endothelium play critical roles in the initiation and progression of atherosclerosis. Resveratrol, a polyphenolic constituent of red wine, has antioxidant effects and may against endothelial injury. In this present study, we examined whether resveratrol protect the oxidized LDL-induced vascular endothelial dysfunction.
**Materials and methods:** Primary human umbilical vein endothelial cell cultures (HUVECs) were treated with oxLDL under cytotoxic and non-cytotoxic concentration to explore the protective effect of resveratrol. Cytotoxicity of oxLDL on HUVECs was studied by measuring lactate dehydrogenase (LDH) release, methylthiazol tetrazolium (MTT) and sustained peak of cytosolic calcium. Effects of oxLDL on apoptotic cell death and oxidative stress were characterized by TUNEL stain and production of reactive oxygen species (ROS), respectively. Furthermore, oxLDL-induced IL-8 production and eNO synthase impairment were investigated by RT-PCR. Surface expression of adhesion molecules were determined by flow cytometry.
**Results:** Resveratrol significantly rescued the cytotoxicity induced by oxLDL (200 µg/ml) appeared by TUNEL stain and also by MTT assay (53 ± 3% vs. 103 ± 3%, compared with control, $P<0.05$). We also found that resveratrol decreased oxLDL-induced intracellular calcium rise from 191 ± 7 nM to 95 ± 15 nM ($P<0.01$), and reduced ROS generation from 4.5-fold to 2.2-fold ($P<0.01$). Additionally, resveratrol inhibited oxLDL-induced surface expression of adhesion molecules, ICAM, VCAM, as well as E-selectin (175 ± 22% to 116% ± 3% ; 244 ± 16% to 158% ± 2% and 260 ± 35% to 160% ± 11%, respectively, compared with control, all $P<0.01$). By RT-PCR, our data revealed that resveratrol decreased oxLDL-induced IL-8 production from 1.5-fold to 1.3-fold, and ameliorated the suppression of NO synthase (79% and 117% respectively, compared with control, $P<0.01$) under non-cytotoxic concentration (100 µg/ml).
**Conclusions:** We demonstrated that resveratrol protect endothelial cells against oxLDL-induced apoptosis by suppressing the increase in ROS, intracellular calcium, cytokine production, expression of adhesion molecules and ameliorated the suppression of eNO synthase.

## 1280

**Serum levels of fibronectin and endothelin-1 in obese and non-obese patients with Type 2 diabetes mellitus**

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**Background and aims:** Endothelial dysfunction is implicated in the pathogenesis of metabolic syndrome and diabetic complications. Cellular fibronectin is an endothelium-derived protein involved in subendothelial matrix assembly. Endothelin-1 is the potent endothelium-derived vasoconstrictive and proliferative factor. However, an association between fibronectin and endothelin-1 production and obesity in diabetic patients remains not fully understood. Therefore, the aim of this study was to investigate serum levels of fibronectin and endothelin-1 in obese and non-obese patients with type 2 diabetes.
**Materials and methods:** We studied 48 patients with type 2 diabetes – 24 with obesity (age: 56.8 + 2.2 years, BMI: 35.3+0.8 kg/$m^2$; waist/hip ratio (WHR) – 0.98 + 0.03; data everywhere are presented as mean+ SEM), 24 patients without obesity (age: 52.1 + 1.6 years, BMI: 24.2 + 0.8 kg / $m^2$;

WHR-0.88+0.02), and 40 non-diabetic subjects - 20 obese (age: 52.8+2.1 years, BMI: 33.9+0.6 kg/m$^2$; WHR - 0.89+0.02), and 20 non-obese (age: 47.4+1.4 years, BMI: 24.0+0.6 kg/m$^2$; WHR - 0.84+0.02). Serum fibronectin, endothelin-1 levels were measured by immunoenzyme assay. Statistical analysis was performed by Student's paired and Pearson's correlation tests.
**Results:** We found an increase of serum fibronectin, endothelin-1 levels in patients with diabetes compared to control subjects either in obese and non-obese persons. In obese subjects fibronectin serum levels were - 399.8+23.94 and 267.41+45.59 pmol/L ($p<0.001$), endothelin-1 - 8.09+0.51 and 5.8+0.45 pmol/L ($p<0.001$) in those with and without diabetes, respectively. In non-obese subjects fibronectin levels were 335.76+58.09 and 226.81+37.46 pmol/L ($p<0.01$), endothelin-1 - 6.67+0.38 and 4.38+0.31 pmol/L ($p<0.01$) in those with and without diabetes, respectively. Moreover, it was a significant elevation of serum fibronectin and endothelin-1 levels in those with obesity compared to non-obese subjects either in people with or without diabetes mellitus. In obese patients with diabetes it was significant correlation between fasting glucose, BMI, on one side, and fibronectin levels, on the other side ($r=0.62$, $p<0.05$ and $r=0.78$, $p<0.05$, respectively) and endothelin-1 levels ($r=0.51$, $p<0.05$ and $r=0.68$, $p<0.05$, respectively). Also, in this group we found significant correlation between fibronectin and endothelin-1 serum levels ($r=0.48$, $p<0.05$).
**Conclusion:** The revealed changes of fibronectin and endothelin-1 serum levels could reflect an endothelial dysfunction in type 2 diabetic patients, which is the most pronounced in those with obesity. Hyperglycemia and obesity appear to be significant factors contributing to elevation of fibronectin, endothelin-1 production in patients with diabetes.

## 1281

**Intermittent high glucose induced apoptosis in HUVECs: the role of mitochondrial superoxide production**
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**Background and aims:** It is now well established that long exposure to hyperglycemia typical of the diabetic condition lead up to micro and macrovascular complications. Damage mechanisms are not yet fully understood but several evidences show that oxidative stress generation is one of the key steps of such alterations. Many papers show the tight connection between high glucose, oxidative stress and apoptosis, especially in the high oscillating glucose condition typical of the diabetic condition.
**Materials and methods:** Primary cultures of human umbilical vein endothelial cells were grown for 14 days. Three groups were formed each one receiving the following fresh media every 24 h: 1) continuous normal glucose medium (5 mM); 2) continuous high glucose medium (20 mM); 3) normal and high glucose media alternating every 24 h. MnTBAP, TTFA and Cu/Zn SOD, were added, separately, to culture media. 8OHdG, Nitrotyrosine, Caspase 3 activity and expression and Bcl-2 expression levels were evaluated at the beginning of the experiment, after 7 and 14 days.
**Results:** High glucose, especially in the oscillating condition, causes oxidative stress with augmented values of Nitrotyrosine and 8OHdG. High glucose and high oscillanting glucose conditions showed also an augmented expression and activity of Caspase-3 and a specular lowered Bcl-2 expression. The inhibitor added to the colture media lead to a normalization of the apoptotic parameters and of the oxidative stress markers considered.
**Conclusions:** Our data show that high glucose and high oscillating glucose injure endothelial cell in colture causing oxidative stress and apoptosis. The normalizing effect of MnTBAP, TTFA and Cu/Zn SOD shows that even in the oscillating high glucose condition, damage is caused by the high free radical production at the mitochondrial transport chain level.

# PS 135
# Pharmacologic effects on endothelial dysfunction

## 1282

**Preventive effects of acarbose on acute, hyperglycemia-mediated endothelial dysfunction in patients with impaired glucose tolerance**
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**Background and aims:** Patients with impaired glucose tolerance are at increased cardiovascular risk. The first functional vascular alteration detectable in that patients is transient endothelial dysfunction, by means of reduced endothelium-dependend vasodilation, in the postprandial phase. This phenomenon, representing a very early step in atherogenesis, seems to depend on postprandial hyperglycemia. Interventional studies on that, however, have not been performed yet. The aim of our study was to investigate whether selective reduction of hyperglycemia, induced by a carbohydrate load, with acarbose (a disaccharidase-inhibitor) influences the concomitant endothelial dysfunction in patients with impaired glucose tolerance.
**Materials and methods:** In a randomised, double-blind, placebo-controlled, cross-over study the effect of acute administration of 200 mg acarbose was investigated in 28 subjects (17 males, mean age 55 ± 9 years) with diagnosed impaired glucose tolerance. Flow-mediated dilation (FMD) of the brachial artery was determined by high resolution ultrasound as a measure of endothelial function before as well as 2 and 3 hours after ingestion of 100 mg saccharose. Asymetrical dimethylarginine (ADMA) was measured by HPLC. Statistical evaluation was done by ANOVA (incl post-hoc tests) or paired t-test respectively. All data are mean±SD, level of significance was $p<0.05$.
**Results:** In the entire population a highly significant negative correlation was observed between the changes of blood glucose and FMD ($r=-0.416$, $p=0.0018$) 2 hours after ingestion of saccharose. At 3 hours, neither blood glucose nor FMD were different from baseline. Both, changes of blood glucose (11.0 ± 4.5 vs. 35.6 ± 7.5 mg/dl, $p=0.0007$) as well as of FMD (0.45 ± 0.32 vs. -0.77 ± 0.46 %, $p=0.046$) were significantly lower after administration of acarbose. Subgroup analysis revealed that the effect of acarbose with regard to both, reduced postload hyperglycemia (23.4 ± 5.4 vs. 67.7 ± 6.9 mg / dl $p=0.0001$) as well as decline of FMD (0.36 ± 0.25 vs. -1.94 ± 0.49 %, $p=0.0016$) was restricted to those subjects with an increase of blood glucose above the median increase of glycemia (34.5 mg/dl) after administration of placebo. No changes of plasma ADMA were observed during our experiments.
**Conclusion:** Selective reduction of postload hyperglycemia with acarbose is able to prevent postload endothelial dysfunction. Thus, our data clearly demonstrate that acute hyperglycemia causes endothelial dysfunction in subjects with impaired glucose tolerance.

## 1283

**Spironolactone worsens endothelial function and heart rate variability in patients with Type 2 diabetes**
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**Background and aims:** Diabetes is an area, like cardiac failure, where angiotensin II (AII) withdrawal has proven to be of particular value. However, the adverese effects of aldosterone are known to be very similar to those of AII. Thus it is logical in diseases where AII withdrawl has proven to be beneficial to consider blockade of aldosterone.
In patients with heart failure additional aldosterone blockade has proven to be beneficial in terms of morbidity and mortality; this is thought to be due, at least in part, to an improvement in endothelial function and heart rate variability. We postulated that aldosterone blockade with Spironolactone might also have beneficial effects on endothelial function and heart rate variability in diabetic patients.
**Materials and methods:** Endothelial function was assessed by forearm venous occlusion plethysmography in 42 patients with type II diabetes after 1 month treatment with Spironolactone or placebo allocated in a randomised double blind trial. We also assessed heart rate variability, haemoglobinA1C (HbA1C) and plasma AII levels at the end of each treatment period.

**Results:** When compared to placebo, Spironolactone significantly decreased forearm blood flow response to acetylcholine by 43.22 ± 14.21% (p=0.003). Spironolactone also worsened heart rate variability parameters; RMSSD decreased by 1.99 ± 0.93 ms (p=0.03), low frequency (LF) normalised power increased by 2.00 ± 0.91nu (p=0.03), high frequency (HF) normalised power decreased by 1.98 ± 0.94nu (p=0.04) and LF:HF ratio increased by 0.40 ± 0.19 (p = 0.04). HbA1C and AII increased during Spironolactone treatment by 0.26 ± 0.07% (p = 0.001) and 8.12 ± 1.94 pg / ml (p < 0.000), respectively.
**Conclusion:** Spironolactone worsened endothelial function and heart rate variability in this population of type II diabetics, this may be due to the worsening in glycaemic control and increasing angiotensin II levels seen during spironolactone treatment.
*Supported by: British Heart Foundation*

## 1284

**The effect of hypolipidemic treatment with statin or fibrate on microvascular reactivity in Type 2 diabetic patients with hyperlipidemia**
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**Background and aims:** Hypolipidemic therapy leads to a significant decrease in the incidence of macroangiopathic complications in diabetic patients as was demonstrated in large trials. However, the effect of hypolipidemic agents on microcirculation is still not clear. The aim of this study was to compare the effect of treatment with simvastatin and fenofibrate on microvascular reactivity in Type 2 diabetic patients with hyperlipidemia.
**Materials and methods:** Twelve Type 2 diabetic men and eight women with hyperlipidemia were randomized into two groups by ten patients. First group was treated with 20 mg of simvastatin daily for three months and then the wash-out period of two months was followed by three months of treatment with 200 mg of micronized fenofibrate daily. Second group was treated with the same design but with the reversed order of hypolipidemics (fenofibrate first). Then the treatment was maintained for one year in each group. Patients were examined and blood samples taken before and after treatment with respective hypolipidemic agent and finally microvascular reactivity was measured after one year of therapy. Microvascular reactivity was evaluated by laser-doppler perfusion monitoring during post-occlusive reactive hyperemia (PORH) and thermal hyperemia (TH) on Periflux PF4001 instrument (Perimed, Sweden). Maximal perfusion during PORH and TH (PORHmax, THmax) expressed in perfusion units [PU] was recorded and velocity of perfusion increase (PORH/t, TH/t [PU/s]) and the percentage increase of perfusion (PORH%, TH% [%]) were calculated and compared for each treatment. Paired t-test was used for statistical analysis.
**Results:** A statistically significant decrease in parameters of lipid metabolism after treatment was observed as supposed. However, significant decrease in microvascular reactivity after both simvastatin and fenofibrate treatment was detected (Table 1).

Table 1. Comparison of parameters of microvascular reactivity during hypolipidemic treatment.

| | TC (mmol/l) | $HbA_{1C}$ (%) | TH max (PU) | PORH max (PU) | TH max/t (PU/t) | PORH max/t (PU/t) | TH% (%) | PORH% (%) |
|---|---|---|---|---|---|---|---|---|
| **Basal** | 6.62 ± 0.50 | 9.0 ± 1.7 | 133.2 ± 45.5 | 1.5 ± 632.3 | 1.44 ± 0.67 | 7.2 ± 6.9 | 1442 ± 535 | 540 ± 155 |
| **Statin** | 5.19 ± 0.57[a] | 9.0 ± 1.8 | 67.0 ± 19.9[b] | 26.4 ± 12.6[b] | 1.07 ± 0.63[b] | 2.0 ± 1.4[b] | 794 ± 296[b] | 419 ± 200[b] |
| **Fibrate** | 5.97 ± 0.49[b] | 9.0 ± 1.7 | 92.0 ± 33.1[b] | 37.2 ± 12.8[b] | 1.18 ± 0.49[c] | 3.1 ± 1.2 | 1199 ± 523 | 220 ± 127[b] |
| **1 year** | - | - | 81.5 ± 37.8[b] | 35.2 ± 17.5[b] | 0.96 ± 0.43[b] | 2.8 ± 1.7[c] | 860 ± 505[b] | 383 ± 264[b] |

*TC - total cholesterol, $HbA_{1C}$ - glycated hemoglobin; statistical significance a - p<0.001, b - p<0.01, c - p<0.05.*

No statistically significant relationship was found between microvascular reactivity and lipid concentrations or diabetes control.
**Conclusion:** A significant decrease in microvascular reactivity was found in this study after both simvastatin and fenofibrate treatment. The decrease was more pronounced after simvastatin therapy and was not related to changes in lipid concentrations or to metabolic control of diabetes. Furthermore, significant changes in microvascular response were persistent even after one year of hypolipidemic treatment. It is not clear whether microvascular reactivity in Type 2 diabetic patients with hyperlipidemia is influenced by direct effect of hypolipidemic medication on microvasculature or by the reparation processes after improvement of hyperlipidemia. Further research will be necessary to test these hypotheses.
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## 1285

**Pioglitazone improves endothelial dysfunction in metabolic syndrome patients with impaired glucose tolerance**
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**Background and aims:** Insulin resistance (IR) and hyperglycemia are associated with endothelial dysfunction that is attributable to oxidative stress and a proinflammatory state and may contribute to development of atherosclerosis(AS). Thiazolidinediones (TZDs) have been shown recently to exert positive effect on endothelial function and attenuate atherosclerosis formation. In addition, TZDs also possess antioxidant and anti-inflammatory properties. We conducted the present study in patients with metabolic syndrome (MS) who have impaired glucose tolerance (IGT) to determine the effect of the TZDs compound pioglitazone on endothelial function and identify its possible mechanism.
**Materials and methods:** 22 MS patients (13 woman and 9 man, age: 52 ± 10 yr, body mass index: 26.3 ± 2.5 Kg/m$^2$) with IGT and no clinical manifestations of macrovascular disease were treated with pioglitazone for 4 months. High-resolution ultrasound images were used to measure the flow-mediated dilation (FMD, endothelium-dependent) and nitroglycerin-induced dilation (NID, endothelium-independent) in the brachial artery. FMD was defined as the maximum percentage increase in vessel diameter during reactive hyperemia and NID was calculated similarly. We investigated oxidative stress by measuring serum concentrations of malondialdehyde (MDA) and tripeptide glutathione(GSH). High-sensitivity C-reactive protein (hs-CRP) was also monitored as a marker of a systemic inflammation.
**Results:** The FMD was improved significantly (means ± SE 6.98 ± 3.34% *vs.*10.00 ± 2.18%, P <0.01) and the NID was not changed (23.28 ± 4.59% *vs.* 24.16 ± 3.18%, P=0.450) after treatment. Pioglitazone therapy also resulted in a significant decrease in fasting insulin (FINS) level (17.66 ± 6.58μU/mL *vs.*11.89 ± 2.58μU/mL, P<0.01). Furthermore, the level of MDA reduced markedly (4.51 ± 1.27 nmol/mL *vs.*3.86 ± 1.44 nmol/mL (P<0.01) ), whereas serum GSH concentration had a moderate increase (216.32 ± 29.36 mg / mL *vs.* 228.31 ± 29.13 mg / mL, P = 0.085). And hs-CRP was significantly lower posttreatment compared to pretreatment [ median (range) 3.60 mg / L (0.1 - 7.54 mg / L) *vs.* 0.48 mg / L (0.1 - 4.07 mg / L), P<0.01]. We found that the improvement of FMD was strongly associated with the changes in GSH and CRP concentrations (r=0.469, P=0.032 and r=-0.581, P=0.011, respectively). And the FMD increase was not correlated with the decreases in MDA and FINS levels.
**Conclusion:** Pioglitazone treatment for 4 months improved endothelial function in MS patients with IGT. This improvement was related to attenuations of oxidative stress and inflammation in vasculature.

## 1286

**Procoagulant changes and vascular endothelial damage in preeclamptic diabetic pregnancy**
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**Background and aims:** Diabetes mellitus is one of the commonest complications of pregnancy in which the incidence of preeclampsia is increased about two-folds. The aim of this study was to characterize the nature of coagulopathy and vasculopathy in preeclamptic diabetic pregnancy.
**Materials and methods:** One hundred and twenty six hospitalized pregnant women in third trimester were recruited in four divided groups [Group 1: 28 healthy pregnant women (HP), Group 2: 32 diabetic pregnant women (DP), Group 3: 40 pregnant women with preeclampsia (PE) and Group 4: 28 pregnant women with preeclampsia and diabetes (DPE)]. Their serum glucose was measured by glucose oxidase method, HbA1c by the Variant hemoglobin testing system using dedicated HPLC, serum C-peptide by an chemiluminescence based ELISA technique, platelet aggregation by optical method, plasma fibrinogen by clotting method, antithrombin III (AT III) by amidolytic method and von Willibrand factor (vWf) was measured by radial immunodiffusion technique. All the results were expressed as median (range).
**Results:** The subjects in all the groups were found to be well controlled both in terms of PE and DM. Platelet aggregation (%) in HP was 86.25 (63–108).

The other 3 groups showed lower values compared to HP [DP 75.0 (51 – 105); PE 71.87 (50–101); DPE 76.0 (46–99)], but the difference was significant only in case of HP vs PE ($p<0.001$). Plasma fibrinogen (mg/dl) tended to be higher in PE [457 (148–595)], DP [403 (60–660)] and DPE [350 (170–560)] as compared to HP [300 (92–650)], but the difference did not reach statistical significance. The level of vWf (IU/ml) was 2.12 (1.05–3.29) in DPE, 2.04 (1.05–3.33) in DP and 2.09 (1.02–2.69) in PE as compared to HP 1.92 (1.26–3.31). The mean ± SD level of AT III (%) was 107.44 ± 8 in DPE, 97.72 ± 11 in HP, 99.93 ± 12 in DP and 102.17 ± 9 in PE. A significant association was found between platelet aggregation and mean blood pressure in preeclamptic group ($p<0.001$), but this association was not observed in other groups. A significant association was also found between vWf and C-peptide in healthy pregnant women ($p<0.05$).
**Conclusion:** Since control of BP and blood glucose is associated with a control of most of the relevant markers, the reported coagulation abnormalities in preeclampsia and diabetes mellitus (increased procoagulant change and decreased anticoagulant change) seems to be secondary in nature in both the conditions. The vascular endothelial damage, as reported in preeclampsia and diabetes mellitus, is also secondary in nature. The coagulopathy and vasculopathy in preeclampsia seems to be related to some primary defects associated with hypertension in PE and hyperglycemia in DM.

# PS 136

## Pathogenic mechanisms: other

### 1287

**Development of a human macrophage model of atherosclerosis and the characterisation of a PPARγ and LXRα ligand**
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**Background and aims:** Accumulation of macrophages and the subsequent build up of cholesterol in arteries leads to foam cell formation and atherosclerotic plaques. Activation of PPARγ using specific agonists at high concentrations has been reported to increase LXR-regulated cholesterol efflux from cells, and so has possible implications in the treatment of atherosclerosis. The aim of this study was to develop a human macrophage model for cholesterol accumulation and investigate the effects of compounds on both cholesterol efflux and regulation of PPARγ and LXRα target genes.
**Materials and methods:** The human monocyte/macrophage cell line THP-1 was cultured in medium containing 10% FCS and differentiated into macrophages using PMA. After differentiation, medium was changed to serum-free medium. Cells were labelled with 0,4 μCi/ml $^{14}$C-cholesterol and treated with ACAT inhibitor CL-277,082 and reference compounds Rosiglitazone, a selective PPARγ agonist, and T0901317, a selective LXR agonist. Specific efflux was induced by adding ApoA1 as a cholesterol acceptor and efflux was quantified by taking aliquots of supernatant and measuring the radioactivity. From the cell lysate total cellular radioactivity was measured. In parallel, RNA was extracted from identically treated non-radiolabelled cells and Taqman technology was used to determine expression levels of PPARγ and LXR-regulated genes.
**Results:** In differentiated THP-1 cells, the inhibition of cholesterol ester formation by an ACAT inhibitor increased the intracellular free cholesterol pool. As a consequence, oxysterols accumulate, and act as endogenous ligands for the LXRs. Both oxysterols as well as the synthetic LXR agonist T0901317 stimulated the LXRα autoregulatory loop as well as the whole ABCA1 pathway of reverse cholesterol transport. The effects of T0901317 and oxysterols were additive with respect to both gene expression and cholesterol efflux. Dose-dependent upregulation of both ABCA1 and another LXRα target gene, SREBP-1 c (involved in triglyceride synthesis), was seen in cells treated with T0901317. While treatment of THP-1 macrophages with Rosiglitazone led to dose-dependent regulations of the PPARγ target genes CD36 and SR-A, it showed no significant upregulation of LXRα and the ABCA1 pathway, and no increase in cholesterol efflux was measured.
**Conclusion:** While in the human THP-1 macrophage cells, both LXRs and PPARγ are present and functional with respect to target gene regulation, only a direct activation of LXR using synthetic or endogenous ligands resulted in a marked increase in reverse cholesterol transport. In this macrophage system, Rosiglitazone displayed no significant activation of the LXR-ABCA1 pathway, consistent with the work of other groups. There are discrepancies however regarding the mode of action of PPARγ, but this may be due to different experimental conditions or phenotypic differences in macrophages derived from different sources.

### 1288

**Increased platelet activation associated with impaired glucose tolerance is improved by acarbose**
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**Background and aims:** Increased platelet activation is frequently described in patients with diabetes mellitus and is at least partially responsible for the unfavorable prognosis of these patients. Recent data show that already in the pre-diabetic state individuals with insulin resistance and/or impaired glucose tolerance (IGT) have a markedly increased cardiovascular risk. Therefore, in the present study, we investigated whether IGT is associated with platelet activation.
**Materials and methods:** Blood samples were collected from young obese Zucker rats, an established model of IGT, at baseline, 30, 60 and 120 minutes after oral application of sucrose (4g/kg). Platelet activation was investigated by measuring platelet-binding of fibrinogen and platelet surface-expression of P-selectin by flow cytometry and compared with lean Zucker rats.

**Results:** In lean Zucker rats, acute sucrose load induced enhanced fibrinogen-binding and P-selectin surface-expression after 30 and 60 minutes (fibrinogen: basal: 13.2 ± 0.9; 30': 16.9 ± 0.6, p<0.05 vs. basal; 60': 18.7 ± 0.3, p<0.01 vs. basal; P-selectin: basal: 29.9 ± 5.2; 30': 69.5 ± 1.8; 60': 84.5 ± 11.9, all p < 0.05 vs. basal). Acute co-administration of acarbose (10 mg / kg) prevented sucrose-induced platelet activation in lean Zucker rats.
In obese Zucker rats, fibrinogen-binding and P-selectin surface-expression were already markedly increased under baseline conditions (fibrinogen: 19.0 ± 0.6; P-selectin: basal: 82.5 ± 6.1). In these animals we also observed an increased platelet-leukocyte adhesion. Treatment with acarbose (12 mg / kg / d in chow) for 7 days significantly reduced basal platelet activation in obese Zucker rats (fibrinogen: 12.7 ± 0.3, p<0.01 vs. placebo; P-selectin: 62.2 ± 4.5 p<0.05 vs. placebo).
**Conclusion:** Acute sucrose ingestion induces platelet activation within 30 minutes and can be prevented by co-administration of acarbose. Already in the prediabetic state of IGT, platelets are markedly activated which may contribute to the increased cardiovascular risk associated with IGT. The positive modulation of platelet activation by acarbose may at least in part explain the reduction of cardiovascular events reported in patients with IGT in the STOP-NIDDM trial.
*Supported by: a grant from Bayer*

## 1289

### Activation of coagulation factor IX with new enzyme discoverd on erythrocyte membranes in diabetes

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**Background and aims:** Cerebral infarction, myocardial infarction and peripheral vascular disease are common causes of death in diabetic patient. Main causes of these complications are thought due to increased intrinsinc coagulation activity. We have discovered new enzyme that activate factor IX on erythrocyte membrane in normals. In this time, we investigated about these enzyme on erythrocyte membrane in diabetes.
**Materials and methods:** Blood was obtained from human adult volunteers including normal (N: n=24) and diabetic patients (DM n=43). Platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared by centrifuging the blood. Washed RBCs were prepared by repeated resuspension in HEPES buffer. Fluorescent observation of activated coagulation factor IX was measured about supernatant of red cell lysate with a photo microscope with epifluorescent optics (Model AX70; Olympus, Tokyo). And determination of the onset time of coagulation was monitored using a damped oscillation rheometer (Kawakami et al: Biorheology 32:521, 1995). Change in logarithmic damping factor (LDF) was monitored after adding $CaCl_2$ solution to anticoagulated blood sample.
**Results:** The average of blood glucose levels and HbA1 c levels in diabetic patients were 184.5 mg/dL and 8.1%, respectively. The onset time of whole blood coagulation is significantly shorter in DM than in N (25.9 min V.S. 35.7 min). And the onset time of PFP with RBC is also shorter in DM than in N (35.9 min V.S. 45.9 min). Fluorescent observation of activated coagulation factor IX is higher in DM than in N (13.3 V.S. 8.3). In diabetics with nephropathy, onset time is shorter than in diabetics without nephropathy. Fluorescent level was also higher in diabetics with macroangiopathy or foot gangrene. In diabetics with high HbA1 c levels (>8.0%) showed shortened onset time and high fluorescent level.
**Conclusion:** In this paper, we revealed that factor IX-activation enzyme was located on the RBC membrane form diabetes. Factor IX activating enzyme may be a serine protease a d the enzyme activity may be identical with that present of RBC membranes in another study. These data suggests RBC may play inportant role in developping angiopathy in diabetes with its blood coagulation activity.

## 1290

### Insulin resistance and major plasma cations in diabetic and nondiabetic gestational hypertension

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**Background and aims:** Insulin resistance is implicated as a converging point in the natural history of preeclampsia (PE)and pregnancy induced hypertension (PIH), two common complications of pregnancy. There is a well established role of the major cations ($Na^+$, $K^+$, $Ca^{2+}$and $Mg^+$) in modulating insulin secretion and sensitivity and there is a substantial genetic, environmental and nutritional heterogeneity known to exist for insulin secretion and resistance as well as for different types of gestational hypertension. We have investigated the relative contribution of insulin secretory dysfunction or resistance and involvement of major plasma cations in Bangladeshi PE and PIH cases – with and without diabetes mellitus (DM) – a known condition of insulin resistance.
**Materials and methods:** Six groups of pregnant women (aged 20–35 years, primigravida and at third trimester of pregnancy) were investigated - PE (n= 17), PE with DM (DPE, n=12), PIH (n=19), PIH with DM (DPIH, n=15), nonhypertensive healthy pregnant women (HP, n =32) and diabetic pregnancy (DP, n=27). PIH and PE were diagnosed by standard criteria, proteinuria was measured by urinary protein-creatinine ratio, $HbA_1C$ by HPLC and plasma C peptide was measured by fluorimetric ELISA. Insulin secretory capacity (HOMA B%) and insulin sensitivity (HOMA S%) were assessed by Homeostasis Model Assessment (HOMA).
**Results:** From the absolute values of C-peptide and from the HOMA analysis both PIH and PE groups showed similar degree of insulin resistance and there was rather a hypersecretory response from the b-cells. [Plasma C-peptide, nmol/l, M±SD 1.07 ± 0.62 in PE, 0.96 ± 0.60 in PIH and 0.64 ± 0.39 in HP, p<0.02–0.001); HOMA B: 217 (53–553) in PE, 193 (104–402) in PIH and 140 (60–725) in HP; p=0.037 for HP vs PE and 0.035 for HP vs PIH; HOMA S: 49 (17–264) in PE, 58 (0.090–131) in PIH and 86 (25–277) in HP; p=0.018 for HP vs. PE and 0.02 for HP vs PIH]. The plasma C-peptide levels and HOMA S values in the diabetic PE (DPE, and PIH (DPIH) groups were not significantly different from the nonhypertensive diabetic pregnant control (DP) and from each other, but there were significant differences regarding HOMA B with a significant reduction in the diabetic counterparts. Plasma ionized sodium did not show any significant difference between any two of the groups. Potassium showed nearly significant elevation in PE as compared with DP [$K^+$in mmol/l: median (range): 3.9 (3.2–4.9) in HP vs. 4.2 (3.5–6.4) in PE; p<0.07]. Plasma $Ca^{2+}$(mmol/l) had a definite tendency of reduction in PE [875 (700–1000) in DP vs. 850 (620–1071) in PE, p<0.07] and it was significantly reduced in PIH [820 (270–1010), p=0.04)]. The diabetic counterparts of PE and PIH had rising tendency in plasma $Ca^{2+}$ values [980 (740–1130) in DPE, and 890 (760–1220) in DPIH]. Regarding $Mg^{2+}$there was no significant difference between any two of the groups excepting a significant rise in the DPE Group.
**Conclusion:** Insulin resistance is a major pathophysiologic feature of gestational hypertension in PIH and PE in Bangladeshi population. The hypertensive states in pregnancy are associated with a tendency to hyperkalemia and hypocalcemia. Coexistence of diabetes mellitus, however, tends to change serum ionized calcium level in the opposite direction.

## 1291

### Gene expression profiling implicates an impaired adipogenesis in insulin resistance

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**Background and aims:** Insulin resistance is a major predictor of type 2 diabetes. In the present study, we characterized the gene expression profiles of isolated adipose cells of non-obese and non-diabetic insulin-resistant first-degree relatives of type 2 diabetic patients using oligonucleotide microarrays. To verify the microarray findings, expression of genes participating in adipogenesis was quantified by real-time PCR in adipose tissue and in adipose cells isolated from portions of the same biopsies.
**Results:** About 600 genes and expressed sequence tags, which displayed a gene expression pattern of cell proliferation, were differentially expressed in the adipose cells. The expression of several Wnt signaling genes related to the lineage-commitment of precursor cells was reduced in the adipose tissue, including WNT1, FZD1, DVL1, GSK3β, β-Catenin, and TCF1. In addition, several adipogenic transcription factors, C/EBPα, β and δ, PPARγ, and SREBP-1, were also reduced. The expression of adipose-specific proteins related to terminal differentiation, e. g. adiponectin and aP2, was reduced both in the adipose tissue and in the isolated adipose cells. The adipose cells were enlarged in the insulin-resistant relatives and the cell size inversely correlated with the expression of the Wnt signaling genes, adiponectin, and aP2, as well as with glucose disposal rate *in vivo*.
**Conclusion:** Our findings suggest that insulin resistance is associated with an impaired adipogenesis and enlargement of adipose cells. Reduced Wnt signaling gene expression may impair early adipogenic commitment and, hence, adipogenesis.

# PS 137

# Vascular pathogenic mechanisms: cytokines and Toll-like receptors

## 1292

**Subclinical inflammation in central obesity and Type 2 diabetes is related to elevated circulating endotoxin levels and increased expression of TLR 2 and 4 in adipose tissue**

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**Background and aims:** Both central obesity and type 2 diabetes (T2DM) are associated with chronic sub-clinical inflammation and hyperinsulinaemia. Our previous studies indicate that adipose tissue produces pro-inflammatory cytokines in response to hyperinsulinaemia. Furthermore, the activation of the innate immune system may occur via the membrane bound toll-like receptors (TLR) 2 and 4 in adipose tissue. However, to date no study has characterised the cause or mechanism by which the activation of the innate immune response may occur.
**Materials and methods:** We investigated whether T2DM patients have higher circulating endotoxin levels to account for the associated circulating chronic sub-clinical inflammatory response arising from human adipose tissue by (1) measuring endotoxin levels in healthy lean non-diabetic (ND) subjects (Age: 27.4 ± (SD) 5.96 yrs; BMI: 23.1 ± (SD) 3.8 kg/m$^2$) compared with T2DM subjects (Age: 54.2 ± 23.2 yrs; BMI: 38.3 ± (SD) 11.9 kg/m$^2$) using an LAL (gram -ve assay) ELISA; (2) we further examined the protein expression of TLR-2 and TLR-4 from Subcutaneous (Sc) adipose tissue (AT) from non diabetic and T2DM subjects by western blotting (3) and finally we examined TLR protein expression in abdominal Sc and omental AT compared with thigh AT to characterise the TLR expression in central fat depots.
**Results:** From our *in-vivo* studies we determined that T2DM had a 1.7 fold increase in endotoxin levels compared with lean ND subjects (ND: 1.17 ± (SEM) 0.01 IU; T2DM: 1.95 ± 0.1 IU; $p<0.001$; n=12). We also assessed C-reactive protein (CRP) levels, which were low in ND subjects (1.27 ± (SEM) 0.55) and significantly raised in T2DM subjects (13.03 ± 0.92; $p<0.001$). Further TLR-2 protein expression was significantly upregulated in AT from T2DM subjects and obese subjects compared with lean ND subjects (TLR2: ND: 1.00 ± (SEM) 0.1; obese subjects: 1.37 ± (SEM) 0.1; T2DM: 1.53 ± (SEM) 0.19; $p<0.05$; n=6) whilst TLR 4 expression was inversely correlated with TLR2 findings. Depot specific analysis of TLR 2 and 4 determined that the pattern of expression of receptor sub-types revealed high levels in omental and abdominal Sc compared with subcutaneous thigh tissue ($p<0.01$).
**Conclusion:** The present study indicates that T2DM subjects have increased circulating endotoxin levels compared with normal ND subjects. As TLR-2 is the principal mediator of the innate immune response following a bacterial antigen, TLR 4 expression may therefore be reduced. In addition the increased protein expression of TLR 2 receptors in obese and T2DM subjects may initiate the production of pro-inflammatory/insulin resistance related factors from adipose tissue. In conclusion, increased adiposity and insulin resistance/hyperinsulinaemia may cause increased endotoxin levels. This may occur via mechanisms related to increased gut permeability, which may further exacerbate pro-inflammatory factor secretion from adipose tissue contributing to the pathogenesis and progression to T2DM.

## 1293

**LPS & Zymosan mediated activation of Toll like receptors (TLR) as part of an innate immune response in human subcutaneous adipocytes with associated down-regulation of adiponectin secretion and elevated TNF-alpha and IL-6 levels**

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Type 2 diabetes (T2DM) is associated with insulin resistance and chronic sub-clinical inflammation. Pro-inflammatory cytokines produced from adipose tissue are secreted as part of the innate immune response and are also associated with insulin resistance. In contrast, adipocyte derived adiponectin is associated with insulin sensitivity and also has anti-inflammatory properties. Hence fat may represent a site for mediating the innate immune response. We therefore investigated a mechanism for the observed reduction in adiponectin secretion in obesity and T2DM. Our studies: (1) determined the protein expression of components of the innate immune system in human adipocytes (2) and assessed the activation of the innate immune system as a mechanism for the regulation of adiponectin. Subcutaneous adipocytes were isolated from subjects (BMI: 24.7plus/minus (SD)4.9; n=10) and western blotting was performed to determine protein expression. The results indicated that subcutaneous adipocytes expressed several key components of the innate immune pathway (TLR 2 and 4, NFkappaB, IkappaB-alpha and lipopolysaccharide (LPS) binding protein). Furthermore, *in vitro* studies in human subcutaneous adipocytes examined the effects of bacterial (LPS) and fungal (zymosan) agents on the regulation of pro-inflammatory agents (IL-6 and TNF-alpha) as well as adiponectin. These findings showed an increase in secretion of pro-inflammatory cytokines TNF-alpha ($p=0.003$ and $p=0.047$, respectively) and IL-6 ($p=0.003$ and $p=0.002$, respectively) whilst a significant reduction in adiponectin secretion (bacterial: $p=0.003$ and fungal: $p=0.002$) was observed. This response to the antigenic agents also increased the expression of TLR-2, a known mediator of innate immune activity. In summary, adipocytes contain key components of the innate immune system, substantiating the role of adipose tissue in the inflammatory response. Furthermore, subcutaneous adipocytes react to antigenic stimuli by secreting pro-inflammatory factors such as TNF-alpha and IL-6, with an associated reduction of adiponectin secretion. This response may be mediated through TLR-2 and TLR-4.

## 1294

**Interleukin (IL)-6, a paracrine regulator of the adipose tissue, impairs adipocyte differentiation.**

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**Background and aims:** Adipose tissue production of IL-6 is positively related to fat cell size and the interstitial concentrations are 50–100-fold higher than the circulating levels. Thus, IL-6 can exert a paracrine/autocrine regulation of the cells in the adipose tissue. Furthermore, IL-6 is associated with insulin resistance in vivo and it also induces insulin resistance in 3T3-L1 adipocytes, and the liver, by impairing insulin signaling and GLUT-4 expression. In this study, we examined if IL-6 influences the growth and/or differentiation of the adipose cells.
**Materials and methods:** 3T3-L1 cells were incubated with or without IL-6 (20 ng/ml) and the PPARγ-ligand, pioglitazone (1 μM), from the induction of differentiation and for an additional 15 days. Twenty genes related to differentiation and insulin-regulated events were followed from day 1 (after induction of differentiation) to day 15 and analysed with real-time PCR.
**Results:** Most of the genes related to differentiation (adiponectin, fatty acid synthase, resistin, perlipin, C/EBPα, FOXC2) and insulin action (GLUT-4, IRS-1, CAP, LPL) were markedly reduced (~50 %) albeit with different time courses. Surprisingly, aP2 expression was not changed. Addition of the PPARγ-ligand, pioglitazone, restored the expression of several genes while C/EBPα, LPL, GLUT-4, fatty acid synthase, IRS-1 and adiponectin remained suppressed. C/EBPα is important for differentiation and development of the insulin-responsive (glucose transport and insulin signaling) phenotype of the adipose cells. Thus, the reduced C/EBPα may be important for the insulin resistance seen in 3T3-L1 adipocytes following long-term exposure to IL-6. Studies at the protein level showed that IL-6 consistently activated the MAP Kinases Erk 1/2 and reduced the activation of the cyclin D kinase (CDK) inhibitors (p18, p21).
**Conclusion:** These data show that IL-6 impairs the differentiation of the adipose cells during adipogenesis. This dysregulation is associated with reduced expression of several key genes involved in insulin signaling and action like LPL, GLUT-4, IRS-1, FOXC2 and adiponectin and it is probably related to the increased activation of the MAP Kinases and decreased CDK inhibition induced by IL-6. Reduced C/EBPα expression is also expected to contribute to an insulin-resistant phenotype of the adipose cells by impairing the normal insulin-stimulated membrane protein recycling processes.

## 1295

**The role of resistin in the innate immune response: as an acute phase reactant in response to antigenic stimuli and a positive mediator of both IL-6 and TNF-α secretion from human isolated subcutaneous adipocytes**

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**Background and aims:** Chronic sub-clinical inflammation is associated with both type 2 diabetes and cardiovascular complications which is cou-

pled with increased pro-inflammatory markers, such as IL6, TNF-α and C-reactive protein (CRP). Resistin represents one of the latest proteins to be linked with inflammation and insulin resistance in obesity. Adipose tissue has previously been established as a source of circulating resistin, TNF-α and IL-6. As our previous studies demonstrated a correlation between serum resistin and CRP ($p<0.04$; n=79) we investigated a) the effect of antigens that activate the innate immune response on the secretion of resistin and other pro-inflammatory cytokines; b) the effect of resistin in stimulating secretion of other pro-inflammatory cytokines from adipose tissue.
**Results:** Initial studies examined the effect of obesity on resistin protein expression using western blotting. *Ex vivo* abdominal subcutaneous (Sc) adipose tissue protein extracted from lean (BMI: 22.1 ± (SD)2.2; n=7) and obese (BMI: 33.9 ± 6.9; n=7) subjects showed a 3 fold increase in resistin protein expression in obese subjects compared with lean subjects (non-obese: 1.0 ± 0.23; obese: 4.3 ± 0.33; $p<0.01$). Next we examined the effect of antigenic stimuli on resistin secretion in isolated Sc adipocytes. Sc adipocytes were treated (14 hr) with either fungal (zymosan; 30ng) or a bacterial (lipoplysacharide (LPS); 10ng) antigen. Analysis of resistin secretion in response to these antigens indicated that both zymosan and LPS increased resistin secretion (control: 1.24 ± (SE)2.1ng/mL; zymosan: 3.1 ± 0.3ng/mL***; LPS: 2.75 ± 0.4 ng / mL***; ***$p < 0.001$; (n=7)). Additionally, Sc adipocytes were treated with human recombinant resistin alone (30ng/mL), in combination with insulin (10nM/mL) and rosiglitazone ($10^{-8}$M), and rosiglitazone alone. Human recombinant resistin elevated the secretion of IL-6 (control: 1962 ± (SEM)130 pg / mL; resistin 30 ng / mL: 2906.4 ± 297.0pg/mL; $p=0.0026$) and TNF-α compared to control (control: 74 ± (SEM)10pg/mL; resistin 30ng/mL: 249.4 ± 36.5; $p=0.001$), with or without the presence of insulin. Further to this, rosiglitazone reduced this resistin-stimulated rise of both IL-6 and TNF-α secretion from the isolated adipocytes.
**Conclusion:** In conclusion, resistin represents an acute phase reactant to antigenic stimuli and further recruits the pro-inflammatory cytokines IL-6 and TNF-α. These findings explain the close association of resistin to inflammation and other pro-inflammatory cytokines, and could represent a potential role of resistin as a factor to link obesity-mediated inflammation to insulin-resistance.
*Supported by: Diabetes UK*

## 1296

**Effect of acute hyperinsulinaemia on plasma concentrations of selected cytokine antagonists in Type 1 diabetes mellitus**
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**Background:** Several cytokines and growth factors have been implicated in the pathogenesis of diabetic angiopathy. Their ability to generate a biological response in vivo is modulated by specific antagonists and soluble receptors.
**Aims:** The aims of the study were: a) to measure the interleukine 1 receptor antagonists (IL-1ra) and tumor necrosis factor alfa soluble receptors p55 (TNFsr1) and p75 (TNFsr2) in plasma and b) to test their responses to acutely-induced hyperinsulinaemia in type 1 diabetes mellitus (DM1).
**Methods:** Plasma concentrations of IL-1ra, TNFsr1 and TNFsr2 were measured before and at 180 min of hyperinsulinaemic ($1\,mU \cdot kg^{-1} \cdot min^{-1}$) euglycaemic ($5\,mmol \cdot l^{-1}$) clamp in 11 DM1 patients without chronic complications and in 12 weight-, age- and sex-matched healthy controls (C).
**Results:** The basal plasma concentration of IL-1ra was significantly higher in DM1 compared to C (63.25 ± 43.35 vs. 31.79 ± 28.04 pg · $ml^{-1}$; $p<0.05$), similary, the basal plasma concentration of TNFsr1 was higher in DM1 compared to C (986.62 ± 164.51 vs. 778.31 ± 71.69 pg · $ml^{-1}$; $p < 0.01$). We haven't found any significant difference in the basal plasma concentration of TNFsr2 between DM1 and C.
Hyperinsulinaemia didn't significantly change the plasma concentrations of IL-1ra (Δ IL-1ra: 13.36 ± 39.04 vs. 5.36 ± 19.51 pg · $ml^{-1}$), TNFsr1 (Δ TNFsr1: -73.88 ± 273.91 vs. 29.58 ± 92.51 pg · $ml^{-1}$) or TNFsr2 (Δ TNFsr2: -131.04 ± 190.06 vs. -9.66 ± 185.56 pg.$ml^{-1}$) in both DM1 and C.
Furthermore, we found a significant positive correlation between the basal TNFsr1 plasma concentration and a) fasting plasma glucose - mean value 4.82 ± 0.44 mmol/l ($r = +0.4628$; $p<0.05$) and b) HbA1 c - mean value 6.31 ± 1.88 % ($r = +0.7098$; $p<0.01$) in the whole group.
**Conclusions:** DM1 without chronic complications is associated with impaired plasma levels of IL-1ra and TNFsr1 in basal conditions with possible impact on control of cytokine activity. Selected cytokine antagonists and soluble receptors were not affected by acutely induced hyperinsulinaemia.
*Supported by: NB/7517 - 3*

## 1297

**Circadian variation in urinary excretion of TNFα-soluble receptor 1 and TNFα-soluble receptor 2 in Type 1 diabetes mellitus**
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**Background:** Several cytokines including tumor necrosis factor alfa (TNFα) have been implicated in the pathogenesis of diabetic angiopathy. The ability of TNFα to generate a biological response in vivo is modulated by specific antagonists and soluble receptors.
**Aims:** The aims of the study were to evaluate the circadian variations in urinary tumor necrosis factor soluble receptor p55 (TNFsr 1) and p75 (TNFsr 2) excretion in type 1 diabetes mellitus (DM1).
**Methods:** Excretion rates of TNFsr 1 and TNFsr 2 were measured in 16 DM1 patients with normal albumin excretion and normal glomerular filtration rate, and in 11 weight-, age- and sex-matched healthy controls (C). Six 3-hour specimens (I-VI) and one 6-hour overnight specimen (VII) of urine were collected during hospitalisation and controlled dietary intake.
**Results:** The urinary excretions of TNFsr 1 and TNFsr 2 were higher in DM1 compared with C measured during 24 hours (TNFsr 1: 1466 ± 469 v.s. 869 ± 393 pg/min; $p< 0.01$ and TNFsr 2: 1938 ± 900 v.s 1100 ± 513 pg/min; $p< 0.05$) and cross all periods (showen in table: ANOVA; $p< 0.01$)
The circadian variations in TNFsr 1 and TNFsr 2 excretions were significant ($p< 0.001$) and comparable in DM1 and C. The excretions of TNFsr 1 and TNFsr 2 were higher during daytime periods than during night periods both in DM1 (TNFsr 1: 1633 ± 627 v.s 1186 ± 503 pg/min and TNFsr 2: 2135 ± 1014 v.s. 1610 ± 812 pg/min) and C (TNFsr 1: 941 ± 403 v.s 748 ± 436 pg/ml and TNFsr 2: 1205 ± 520 v.s. 925 ± 641 pg/ml).
**Conclusions:** DM1 with normal renal haemodynamics is associated with impaired renal excretion of TNFsr 1 and TNFsr 2 with a potential impact on the development of diabetic nefropathy. (Supported by IGA MZ CZ grant VZ/CEZ:L17/98:00023001)

Urinary excretion of TNFsr 1 and TNFsr 2 (pg/min):

| Period | I. | II. | III. | IV. | V. | VI. | VII. |
|---|---|---|---|---|---|---|---|
| TNFsr 1 diabetics | 2121 ± 1264 | 1954 ± 1023 | 1407 ± 513 | 1537 ± 658 | 1146 ± 650 | 1213 ± 488 | 1173 ± 561 |
| TNFsr 1 controls | 1302 ± 1078 | 1302 ± 1078 | 746 ± 479 | 763 ± 554 | 836 ± 428 | 658 ± 461 | 793 ± 602 |
| TNFsr 2 diabetics | 2720 ± 1780 | 2504 ± 1408 | 1888 ± 972 | 1953 ± 1199 | 1609 ± 1070 | 1568 ± 951 | 1631 ± 851 |
| TNFsr 2 controls | 1882 ± 1124 | 1603 ± 973 | 864 ± 479 | 894 ± 746 | 783 ± 379 | 613 ± 506 | 1080 ± 921 |

# PS 138

# Connective tissue, infection and other diabetes-related sequelae

## 1298

**Shoulder pain in patients with diabetes**

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**Introduction:** Frozen shoulder is a poorly understood condition resulting in a painful, stiff shoulder joint, with no known underlying cause. One of the risk factors for this condition is diabetes mellitus (DM), although the nature of this association is not clearly understood. This study was performed in order to determine the prevalence of frozen shoulder in a large cohort of diabetic subjects compared to a non-diabetic population, and investigate correlations between shoulder symptoms and certain aspects of the diabetes history.
**Methods:** Over three months, all patients attending a diabetic clinic and a general medical clinic (controls) were approached and asked to complete a short questionnaire enquiring about presence of shoulder symptoms, type and duration of diabetes, and diabetes therapy. Subjects with symptoms were then interviewed and examined. Those with a history of trauma or inflammatory arthritis were excluded. Further details were taken with regards to diabetes (duration, method of control), and shoulder symptoms (site affected, visual analogue scale, Oxford Shoulder Score). Shoulder external rotation was measured for both shoulders. Blood samples were taken from all diabetic patients to measure HbA1 c.
The data were then analysed, grouping subjects into non-diabetic subjects with and without pain; diabetic subjects without pain; diabetic subjects with pain; and diabetic subjects with pain and stiffness (defined as <30° external rotation).
**Results:** After exclusions, 806 diabetic subjects (49% male, average age 58.7 years) and 213 non-diabetic subjects (40% male, average age 53.5) completed the questionnaire. 21.5% of the diabetic subjects (n=173) and 3.3% of the non-diabetic subjects (n=7) complained of shoulder pain. 5.3% of the diabetic subjects (n=43) and 0.5% of the non-diabetic subjects (n=1) described pain and stiffness. The average HbA1 c in the diabetic subgroup was 8.3 %. There was no significant difference in average HbA1 c between those with or without shoulder pain or stiffness. There was no significant difference in age. The average duration of diabetes for pain-free diabetic patients was 9.4 years. This increased to 11.9 years for those with shoulder pain and to 13.4 years for those with pain and stiffness. Subjects with type 1diabetes constituted 20% of those without pain, 12% with pain, and 16% with pain and stiffness. 54% of diabetic subjects without pain, 52% of those with pain and 64% with pain and stiffness were treated with insulin therapy.
**Conclusions:** There is a higher prevalence of shoulder pain in diabetic subjects (21.5%) than in non-diabetic subjects (3.3%). There is also a higher prevalence of shoulder stiffness and reduced range of motion in the diabetic subgroup (5.3% vs 0.5%). This has not been demonstrated previously and may represent a truer picture of the prevalence of frozen shoulder. Presence of pain and stiffness is related to duration of diabetes. There is a higher use of insulin therapy in those with pain and stiffness, but this may reflect the longer duration of diabetes in this group. This study did not demonstrate any associations between age or glycaemic control and the presence of pain and stiffness in the shoulder.

## 1299

**Risk factors associated with the tropical diabetic hand syndrome, Dar es Salaam, Tanzania: a case-control study**

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**Background:** The tropical diabetic hand syndrome (TDHS) affects diabetes patients in the tropics, and encompasses a localized cellulitis with variable swelling and ulceration of the hands that may progress to fulminant sepsis and gangrene affecting the entire arm.
**Aims:** To characterize the epidemiology and ascertain risk factors associated with TDHS in patients presenting to Muhimbili National Hospital (MNH), Tanzania.
**Material and Methods:** We conducted a case-control study.
Following informed consent, we evaluated and enrolled consecutive TDHS patients attending the MNH diabetes clinic during June 1998 – March 2004 (study period). A case was defined as any adult patient who presented with cellulitis, ulceration, or gangrene of the hand during the study period. Controls were randomly selected diabetic patients without hand symptoms, who were seen during the study period. Recorded data included demographics, type and duration of diabetes, alcohol and tobacco use, precipitating events, clinical course, and outcome.
**Results:** 120 patients met the case-definition: 61 (51%) female and 85 (71%) with type 2 diabetes; 320 controls were selected. Case characteristics were as follows: median age = 52.5 (range 17–89) years; median blood glucose=15.0 (range 3.1–34.8) mmol/L; median duration of diabetes=6 years (range: 2 weeks–34 years); median duration of hand symptoms=7 days (range: 1 day–3 years); median body mass index (BMI)=23.8 (range: 15.1–39.0) kg/m$^2$. All 120 case-patients had hand ulceration: 107 (89%) drained pus, 119 (99%) had Wagner severity scores ≥2, and 24 (20%) had frank gangrene. Precipitating causes included boils (n=16), mild hand trauma (n=15), or small papules (n=12); 31 (26%) cases occurred spontaneously with no apparent precipitating event. Of 76 (63%) patients who underwent surgery, 12 (10%) had amputations. Seven (6%) patients died from overwhelming sepsis. Case- and control-patients were similar for region of residence, presence of micro- or macrovascular disease, and blood glucose levels. Logistic regression analysis yielded the following independent case correlates: African race (p<.0001), female sex (p <.0001), higher median age (p <.05), longer median duration of diabetes (p <.0001), lower BMI (p <.0001), or type 2 diabetes (p <.05). Of 98 patients who had complete healing, 46 (47%) continued to experience long-term neuropathic pain.
**Conclusions:** TDHS appears to be an acute event that is not related to peripheral neuropathy or large vessel disease. Low BMI and female predominance among TDHS cases suggest metabolic dysfunction, malnutrition, or complex socio-behavioral factors might be playing larger roles in the pathogenesis of the condition than was previously thought. Ultimate preventive efforts must include educating at-risk patients on the importance of hand care.

## 1300

**Asymptomatic bacteriuria is a predictor of subsequent hospitalization with urinary tract infection in diabetic adults: The Fremantle Diabetes Study**

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**Background and aims:** There are limited data relating to the prognosis of diabetic patients with asymptomatic bacteriuria (ASB).
**Materials and methods:** We studied 496 non-pregnant adults with type 1 or type 2 diabetes who were participants in an observational study of diabetes in an urban community setting. A single mid-stream urine specimen was taken for aerobic culture and antibiotic sensitivity testing in addition to detailed clinical and laboratory data. ASB was defined as the presence of >$10^5$ colony-forming units/ml of one or two organisms in the absence of symptoms of urinary infection. Patients were followed for a mean (SD) 2.9 ± 0.6 years for hospital admission for urinary sepsis or death via linkage with the Western Australian Data Linkage System.
**Results:** Thirty-six patients (7.3%) had ASB, comprising 33 females (14.4% of females) and 3 males (1.1% of males). Only female gender predicted ASB amongst a range of variables including indices of metabolic control. Twenty-two patients (4.4%) had a subsequent hospital admission for complicated urinary sepsis (CUS), of which CUS was the principal diagnosis in 11 (50%). In a Cox proportional hazards model, ASB was associated with a five-fold increase in risk of hospitalization for CUS as principal diagnosis (hazard ratio (95% confidence interval); 4.99 (1.29–19.26)) and approaching a four-fold increase for CUS as principal or secondary diagnosis (3.74 (1.36–10.24)). ASB did not predict hospitalization for non-urinary sepsis.
**Conclusion:** These data suggest that ASB is not a benign condition in diabetes and that active screening for, and pre-emptive treatment of, ASB may be justified in diabetic females.
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## 1301

**Are patients with Type 1 and Type 2 diabetes at increased risk for development of infections?**

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**Background and aim:** Some studies suggest that patients with type 1 and type 2 diabetes (DM1 and DM2) are at increased risk of developing infections of the skin and mucous membranes (SMI), urinary (UTI) and respiratory tract (RTI). This may be explained by a decreased T-cell mediated immune response in vitro. However, many factors influence the occurrence of such infections and it remains uncertain whether the presence of diabetes is a risk factor independent of other risk factors. Since most DM patients are treated in primary care and guidelines for antibiotic treatment are based primarily on findings from hospital settings, knowledge is required on this relationship to improve the prevention and treatment of infections among this large group of patients. Besides, patients with DM2 regularly visit their general practitioner and infections may be diagnosed earlier compared to those in the general population. We therefore aimed to determine the independent risk of the presence of DM1 and DM2 for the occurrence of SMI, RTI and UTI in general practice.
**Materials and methods:** *Setting and patients.* We conducted a prospective cohort study using computerised medical databases as part of the Dutch National Study in Primary Care (n=300,000). Cohort members aged 18 years or older with DM1 (n=724) and DM2 (n=6,838) were followed up for 12 months and compared with control patients who were diagnosed with uncomplicated hypertension (n=15,153).
*Baseline risk factors.* Possible risk factors that may confound the association between diabetes and outcome included demographic data such as age, gender, urbanisation level and cultural background. Also, data were recorded on relevant co-morbidity including pulmonary, renal, cardiovascular, vaginal, psychiatric, neurologic and thyroid disease and peripheral neuropathy.
*Outcome measures.* A first episode of a physician-diagnosed SMI, RTI and UTI. RTI were subdivided into upper (URTI) and lower (LRTI) infections. SMI were subdivided into bacterial skin infections (BSMI) and mycotic infections (MSMI).
*Statistical analysis.* Multiple logistic regression analysis was used to assess the independent association between DM and outcome measures and data were given as adjusted odds ratios (OR) and corresponding 95% confidence intervals (95% CI).
**Preliminary results:** The mean age of patients with DM1, DM2 and controls was 55 (± 21), 66 (± 13) and 61 (± 13) years, respectively. Corresponding percentages of males were 46%, 46% and 37%. In all, patients with DM1 and DM2 developed statistically significant more often SMI, RTI and UTI compared with controls ($p<0.05$), except for URTI. Results of multivariate analysis will be demonstrated.

## 1302

**Reliable efficacy over time of vardenafil, a potent, selective PDE-5 inhibitor in men with diabetes erectile dysfunction: a retrospective analysis of three Pivotal Phase III Studies**

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**Introduction:** Erectile dysfunction (ED) occurs with higher frequency and is of greater severity in patients with diabetes. Reliability of a pharmacotherapy of ED may improve the desire for therapy and subsequent continuity of treatment. Vardenafil is a potent, selective PDE5 inhibitor developed for the treatment of erectile dysfunction. Here, the reliability and safety of vardenafil were evaluated.
**Patients and methods:** This *post-hoc* study analyzed data from three randomized, double-blind Phase III trials in which men with ED for >6 months received fixed doses of vardenafil 10 mg or 20 mg or placebo as needed for up to 12 weeks. Data are derived from the intent-to-treat (ITT) population; patients must have had at least 2 valid diaries to qualify for this analysis, and were followed up to week 12. Outcomes of interest were diary questions on overall mean vaginal penetration rate (SEP-2) per patient, overall mean erection maintenance rate (SEP-3) per patient, and overall satisfaction with the sexual experience. Data are presented for each outcome as first-attempt success rates and subsequent success rates for those successful at first attempt up to week 12.
**Results:** There were 202/210, 210/215, and 214/220 patients in the placebo, vardenafil 10 mg, and vardenafil 20 mg in the ITT population eligible for analysis. Reliability rates are shown in table.

| | Placebo | | Vardenafil 10 mg | | Vardenafil 20 mg | |
|---|---|---|---|---|---|---|
| | First | Subsequent | First | Subsequent | First | Subsequent |
| SEP2 | 35.1% | 73.5% | 61.0% | 81.3% | 62.1% | 85.3% |
| SEP3 | 22.8% | 62.6% | 47.1% | 77.4% | 47.7% | 80.5% |
| Satisfaction | 19.8% | 54.5% | 41.0% | 73.0% | 43.9% | 72.8% |

The most common drug-related adverse events were headache, flushing, rhinitis, and dyspepsia, were dose-related, transient, and mild-to-moderate in intensity.
**Conclusion:** Vardenafil provides clinical improvement in first and subsequent attempts assessed by penetration, maintenance, and satisfaction rates up to week 12 relative to placebo, in this difficult-to-treat population. Thus, vardenafil provides high reliability in key efficacy parameters important to patients with diabetes and ED in choosing and continuing oral treatment for ED.

## 1303

**Renal localization of transplanted bone marrow-derived stem cells, effect of hyperglycemia**

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**Background and aims:** Recent reports suggest that bone marrow-derived stem cells (BMSCs) may differentiate into different tissues. Transformation of BMSCs into renal cells has also been described. Hyperglycemia is an established cause of nefrotoxicity, but whether a prolonged exposure of the kidney to high ambient glucose might modulate the homing of BMSCs into renal parenchyma is presently unknown.
Aim of this study is to clarify: a) whether transplanted BMSCs localize in renal parenchyma, b) whether prolonged hyperglycemia modulates this phenomenon and finally c) whether BMSCs recruited by the kidney can be rescued and grown in vitro.
**Materials and methods:** Four C57BL/6J mice were studied. Bone marrow was removed by lethal irradiation and the animals were transplanted with 1 million bone marrow cells from a syngenic green fluorescent protein (GFP) mouse. One week after transplant, two animals were made diabetics by injection of streptozotocin and two were used for control purposes.
Two mice were sacrificed 15 days after bone marrow transplant and two after one month.
**Results:** GFP positive cells could be detected in renal sections of recipient mice inside the glomerulus and tubular wall. Diabetic mice showed an increased number of GFP positive cells inside the renal parenchyma. After two weeks of in vitro culture of extracted glomeruli, outgrows of GFP positive cells could be appreciated. Glomerular GFP positive cells, as expected for mesangial cells, stained negative for cytokeratin, CD45, synaptopodin and Von Willebrand factor.
**Conclusion:** Our results show that BMSCs are able to repopulate the kidney and that this phenomenon is amplified by a concomitant hyperglycemia. GFP positive cells grown from cultured glomeruli are viable, rapid proliferating in vitro and morphologically and immunohystochemically similar to mesangial cells. The potential use of BMSCs cells in repairing a damaged kidney remains to be investigated by further morphological and functional studies.

## 1304

**Factors affecting bone mass in diabetic patients: body mass index, diabetes duration, presence of retinopathy and nephropathy**

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**Background and aims:** Bone metabolism and fracture risk is affected by many interacting factors including hyperinsulinism, presence of nephropathy, decreased visual acuity due to retinopathy, and malnutrition in patients with diabetes mellitus. Although there is no specific bone disease attributed to diabetes mellitus, some risk factors specific to the patient can be recognised which have potential influence on bone metabolism. The aim of our

study was to find out factors with potentia! contribution to bone metabolism in patients with type 2 diabetes mellitus.
**Materials and methods:** Patients with history of type 2 diabetes mellitus were recruited randomly for this study. Their demographic characteristics, metabolic status, chronic complications and bone mineral dansitometry of radius, lumbar vertebrae and femur were recorded. As a control group non-diabetic healthy age matched subjects were analysed with respect to bone dansities.
**Results:** A total of 299 type 2 diabetic patients were enrolled for the study. Mean age of the patients were similar in women and men, 62.3 ± 9.2 and 67.2 ± 7.6 years, respectively. Mean diabetes duration and Hb A1 c were 10.2 ± 9.1 years and 7.5 ± 2.5 % , respectively. Mean body mass index was 30.2 ± 5.2 kg/m$^2$. Frequencies of patients with osteoporosis at femur Ward's triangle, femur neck, lumbar vertebrae, distal radius and midradius were 25.3, 5.4, 23.2, 25.5 and 28.8% , respectively. Patients with lumbar, radius and/or femur osteoporosis were older and revealed longer duration of diabetes and lower body mass index (p=0.0001, p=0.0001, p=0.001, respectively). Prevalence of any degree of nephropathy and retinopathy were higher in patients with osteoporosis(p=0.008; p=0.008, respectively). Diabetic men and women revealed similar bone dansities. Male diabetic patients revieled increased frequency of osteoporosis at any site according to age matched healthy male subjects.
**Conclusion:** Increasing age and diabetes duration, low body mass index and presence of any degree of nephropathy and retinopathy are risk factors for osteoporosis in patients with type 2 diabetes mellitus.

## 1305

**Insulin resistance and its modulation by body weight and leptin in pregnancy induced hypertension**
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**Background and aims:** Pregnancy induced hypertension (PIH) is a common complication of pregnancy and it is known to be an insulin resistant condition. We have studied the inter-relationship of insulin secretory capacity, insulin sensitivity and serum leptin in normal weight PIH, obese PIH and healthy control groups (termed as PIH, OPIH and Control groups respectively). The objective was to explore whether insulin resistance in PIH is a function of weight and associated changes in serum leptin.
**Materials and methods:** Thirty PIH (age in year, 24.82 ± 5.4; BMI in Kg/m$^2$, 21.71 ± 1.9, M±SD), twenty two OPIH (age, 26.91 ± 3.7; BMI, 30.75 ± 3.9) and thirty three Control (age, 26.09 ± 4.33; BMI, 22.47 ± 1.5) subjects were studied for their fasting blood glucose (glucose-oxidase), lipids (enzymatic-colorimetric method), fasting insulin (chemiluminescence-based ELISA) and leptin (ELISA). Insulin secretory capacity (HOMA B) and insulin sensitivity (HOMA S) were calculated by homeostasis model assessment.
**Results:** The blood glucose and lipid levels did not differ significantly between any two of the three groups. There was a significant degree of hyperinsulinemia in the PIH group as compared to Control [serum insulin, pmol/l, median (range): 47.2 (13.9–318.1) in control vs 89.9 (32.0–361.0) in PIH group; p <0.002)]. There was also a rise of serum insulin in the OPIH group; but, the rise was not as pronounced as in the PIH group [69.8 (18.8–330), p<0.04 in PIH vs OPIH)]. A parallel finding was observed in HOMA B [%, 111 (14–529) in Control, 190 (71540) in PIH and 129 (43–245) in OPIH, p<0.001 in Control vs PIH and p<0.037 in Control vs OPIH]. The HOMA S value in PIH [%, 52 (14–141)] was significantly low (p<0.033) as compared to Control group [88 (88–328)], but the fall in sensitivity was, to some extent reversed in the OPIH group [69 (13–227)]. Serum leptin was significantly higher in PIH group [19.4 (5.8–75.2)] as compared to Control [ng/ml, 11.3 (3.4–38.38)]. In contrast to the observations in cases of HOMA B and HOMA S, leptin showed a continued rising trend in the OPIH group [29.5 (4.5–73.9)]. On multiple regression analysis serum leptin was found to be inversely correlated with HOMA B.
**Conclusion:** Pregnancy induced hypertension is an insulin resistant condition which is modulated by serum leptin levels at higher ranges of body weight due to the inhibitory effect of the hormone on insulin secretion.

## 1306

**Non-alcoholic fatty liver disease in Japanese Type 2 diabetes mellitus: relation to regional adiposity, fatty acids and iron deposit**
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**Background and aims:** The current study was undertaken to examine metabolic and body composition correlates of Non-Alcoholic Fatty Liver Disease (NAFLD) in patients with type 2 diabetes mellitus (DM). Various laboratory data can be examined, but it is not known what laboratory data are correlated with the presence of fatty liver in type 2 DM or with the fat content of liver.
**Materials and methods:** One hundred Japanese patients with type 2 DM [mean body mass index (BMI): 24.9 +/– 0.5 kg/m$^2$] had body composition assessments of fat mass (FM), visceral adipose tissue (VAT), liver and spleen computed tomography (CT) attenuation (ratio of liver to spleen (L/S)), as well as evaluations of serum iron, ferritin and adiponectin; these assessments were also performed in nondiabetic volunteers.
**Results:** A majority of those with type 2 DM (27.9%) met CT criteria for fatty liver (ratio of liver to spleen<1.0). Fatty liver was most strongly correlated with VAT (P < 0.0001) and Choline esterase. Fatty liver was less strongly but significantly associated with BMI and FM (P < 0.001), but only weakly associated with subcutaneous adiposity and was correlated with body weight. In the patients with severe Type 2 DM (HbA1 c>6.8), iron was significantly correlated with L/S ratio, but was not correlated in those with milder type 2 DM (5.8≤HbA1 c<6.8). Patient with fatty liver (L/S<1.0) had significantly higher iron and ferritin serum concentrations than those without fatty liver. Serum ferritin concentration is correlated with L/S ratio (P<0.0001), VAT (P=0.0012), FM, BMI and body weight. Serum adiponectin was correlated with HDL-cholesterol and VAT/SAT ratio. Significatly, in patients with type 2 DM (HbA1 c>6.0), adiponectin was inversely correlated with ferritin, L/S ratio and VAT.
**Conclusion:** NAFLD is relatively common in obese type 2 DM patients. Iron concentrations are significantly higher in severe type 2 DM patient, and serum ferritin concentrations are correlated with L/S ratio and VAT. Adiponectin was inversely correlated with L/S ratio. Therefore levels of serum iron, ferritin and adiponectin can serve to predict the development of NAFLD without the evaluation of L/S ratio by CT.